Dissertation

ON

CHRONIC KIDNEY DISEASE AND PLASMA HOMOCYSTEINE LEVEL

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CERTIFICATE

This is to certify that this dissertation entitled " CHRONIC KIDNEY DISEASE AND PLASMA HOMOCYSTEINE LEVEL " is the bonafide original work of **Dr ADARSHA .G.K** in partial fulfillment for the requirements for the M.D General medicine (Branch-1) examination of the Tamilnadu Dr. M.G.R Medical university to be held in April -2015 . The period of the study was from January 2014 to August 2014

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DECLARATION

I **Dr ADARSHA . G.K**. solemnly declare that dissertation titled "CHRONIC KIDNEY DISEASE AND PLASMA HOMOCYSTEINE LEVEL" is a bonafied work done by me at Thanjavur Medical College , Thanjavur during January 2014 to August 2014 under the guidance and supervision of **Professor Dr .S . MANOHARAN , M.D** UNIT CHIEF M3 & M6 Thanjavur medical college ,Thanjavur .

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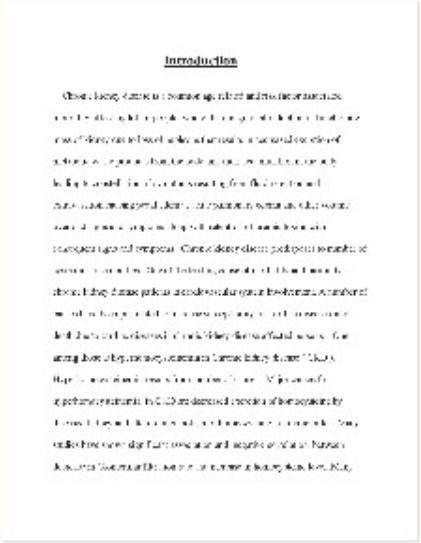
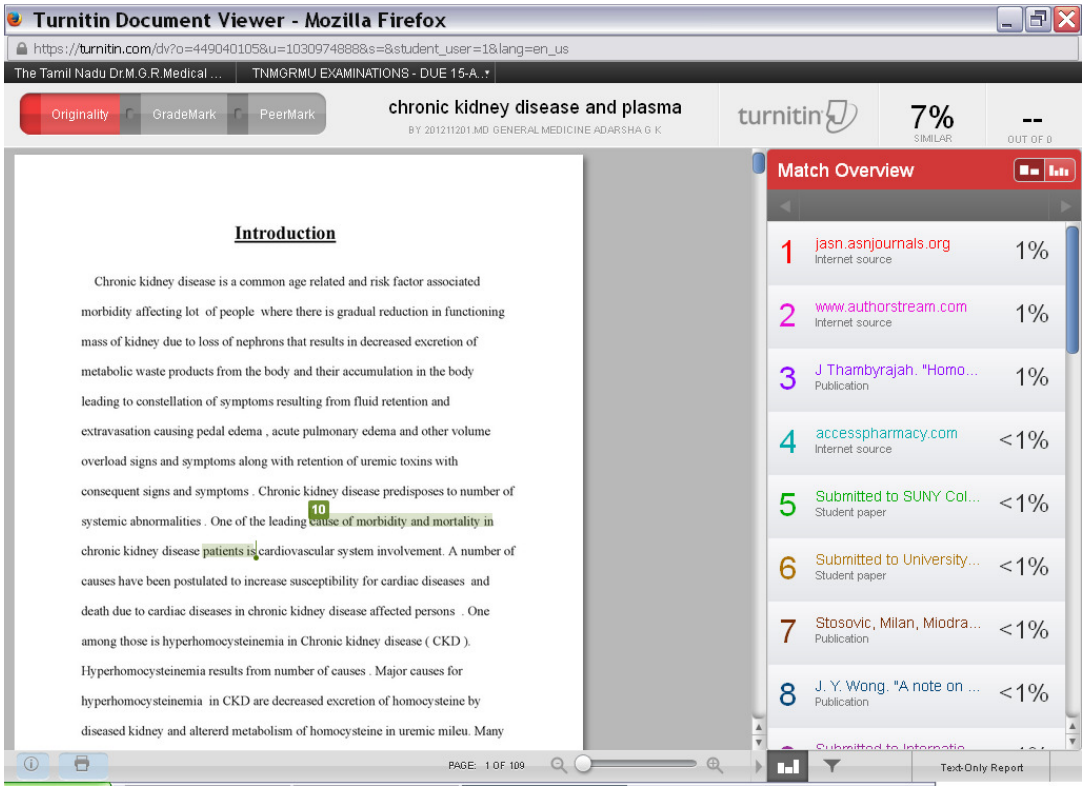


Introduction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Introduction
Chronic kidney disease is a common age related and risk factor associated morbidity affecting lot of people where there is gradual reduction in functioning mass of kidney due to loss of nephrons that results in decreased excretion of metabolic waste products from the body and their accumulation in the body leading to constellation of symptoms resulting from fluid retention and extravasation causing pedal edema , acute pulmonary edema and other volume overload signs and symptoms along with retention of uremic toxins with consequent signs and symptoms . Chronic kidney disease predisposes to number of systemic abnormalities . One of the leading cause of morbidity and mortality in chronic kidney disease patients is cardiovascular system involvement. A number of causes have been postulated to increase susceptibility for cardiac diseases and death due to cardiac diseases in chronic kidney disease affected persons . One among those is hyperhomocysteinemia in Chronic kidney disease (CKD). Hyperhomocysteinemia results from number of causes . Major causes for hyperhomocysteinemia in CKD are decreased excretion of homocysteine by diseased kidney and altererd metabolism of homocysteine in uremic mileu. Many
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LIST OF ABBREVIATIONS

HCY-Homocysteine

CKD –Chronic kidney disease

ESRD – End stage renal disease

MI- Myocardial infarction

NAC –N –acetyl cysteine

PTH-Parathormone

ACE- Angiotensin converting enzyme

ECG –Electrocardiograph

DM –Diabetes Mellitus

SHT –Systemic hypertension

CHRONIC KIDNEY DISEASE AND PLASMA HOMOCYSTEINE LEVEL

ABSTRACT :

Background : . Chronic kidney disease(CKD) is a very common condition result of different etiologies.There are reports in literature indicating that chronic kidney disease leads hyperhomocysteinemia which contributes to increased cardiovascular morbidity and mortalities. Reduction of homocysteine level may help to decrease the cardiovascular morbidity and mortality which are leading cause of death in chronic kidney disease patients

Material and methods : 50 patients with CKD admitted to Thanjavur medical college were selected for study and their fasting plasma homocysteine level were measured. Patients who are having acute kidney injury, who are current smokers,alcohol consumers , who are having liver diseases , who are diabetic are excluded from study. Normal plasma homocysteine level was considered to be below 15 umol/litre .

Observations & Results :Of the 50 patients 37 were males 13 were females . 34 patients were in age range of 41-60 . 36 patients were in stage 5 CKD , 11 were in stage 4 CKD , 3 were in stage 3 CKD . Hyperhomocysteinemia was observed in 78 % of CKD patients . 94.87% had mild hyperhomocysteinemia . End stage renal disease patients had increased prevalence of hyperhomocysteinemia .

There was not much difference in hyperhomocysteinemia in patients who are on dialysis and who are not on dialysis

Conclusion: Hyperhomocysteinemia is highly prevalent in CKD patients.

Key words : hyperhomocysteinemia , Chronic kidney disease , homocysteine

INTRODUCTION

Chronic kidney disease is a common age related and risk factor associated morbidity affecting lot of people where there is gradual reduction in functioning mass of kidney due to loss of nephrons that results in decreased excretion of metabolic waste products from the body and their accumulation in the body leading to constellation of symptoms resulting from fluid retention and extravasation causing pedal edema , acute pulmonary edema and other volume overload signs and symptoms along with retention of uremic toxins with consequent signs and symptoms . Chronic kidney disease predisposes to number of systemic abnormalities . One of the leading causes of morbidity and mortality in chronic kidney disease patients is cardiovascular system involvement. A number of causes have been postulated to increase susceptibility for cardiac diseases and death due to cardiac diseases in chronic kidney disease affected persons. One among those is hyperhomocysteinemia in Chronic kidney disease (CKD). Hyperhomocysteinemia results from number of causes . Major causes for hyperhomocysteinemia in CKD are decreased excretion of homocysteine by diseased kidney and altererd metabolism of homocysteine in uremic mileu. Many studies have shown significant association and negative correlation between decrease in Glomerular filtration rate and increase in homocysteine level .Many studies are ongoing to find out whether

decreasing homocysteine in CKD patients will decrease cardiovascular morbidity and mortality. So in this study an attempt is made to find out association and correlation between decreased renal function and an increase in homocysteine level .

AIMS AND OBJECTIVES

1. To study homocysteine level in patients with Chronic kidney disease

2. To study the association and correlation between decrease in renal function and increase in homocysteine level in chronic kidney disease patients

REVIEW OF LITERATURE

Kidneys are vital organs of human body .Each kidney situated posteriorly behind the peritoneum on each side of the vertebral column [1]. Superiorly they are at the superior border of 12^{th} dorsal vertebra ,inferiorly at the 3^{rd} lumbar vertebra , left is somewhat superior to the right .Left usually little longer and smaller than right and lies closer to the median plane . Each kidney is typically 3cm in anteroposterior direction, 11cm in length, 6cm width .Average weight of human kidney is 150 gm in men and 135 gm in women. Nephrons are functional unit of kidneys .It consists of renal tubule and its associated glomerulus. Each human kidney has approximately 1 million nephrons .There are different types of nephrons .They are nephrons in the cortex and nephrons in Juxtamedullary region. Nephrons in cortex have short loop of Henle where as nephrons in juxtamedullary region have long loop of Henle . In humans 15% of nephrons are juxtamedullary nephrons .

Functions of kidney[2] :

Main work of kidney is removal of waste products produced due to metabolism **.** It does that function through a complex process of glomerular filtration , tubular absorption ,tubular secretion . Glomerular filtration is plasma ultrafiltrate formed due to filtration of blood at glomeruli.

Glomerular filtration rate(GFR) is one of the important indicators of well-being of kidney. As kidney function deteriorates GFR also decreases.

Other important functions of kidney :

1.**Water and electrolyte balance .**

Kidney regulates water content of body and electrolyte content . If kidney is not functioning normally volume overload or dehydration may develop. Electrolyte imbalances like hyponatremia , hyperkalemia and other acid base disturbances may develop in kidney malfunctioning

2. **Endocrine function :**

Kidney produces hormones like erythropoietin which helps in erythropoiesis. Kidneys also produce 1,25-dihydroxycholecalciferol which helps in Vitamin D metabolism and calcium homeostasis in body. In kidney diseases calcium, phosphorous and Vitamin D metabolisms are altered which leads to lot of complications .Kidney produces renin which helps in maintenance of electrolyte in the body and blood pressure control .

CHRONIC KIDNEY DISEASE[3]:

Chronic kidney disease is an entity which encompasses a spectrum of derangement of kidney function in which there is progressive decrease in functioning nephrons and a progressive reduction in glomerular filtration rate. National kidney Foundation (Kidney Dialysis Outcome Quality Initiative) classifies CKD into 6 stages depending on GFR

CLASSIFICATION OF CHRONIC KIDNEY DISEASE

Stage	GFR ml/min per 1.73m2
0	>90*
1	>90#
2	60-89
3	30-59
4	15-29
5	<15

*with risk factors for CKD

#with demonstrated kidney damage (e.g persistent proteinuria, abnormal sediment ,abnormal urine and blood chemistry , abnormal imaging studies)

In contrast to chronic kidney disease chronic renal failure refers to the process of continuing significant irreversible decrease in nephron number

and classically referring to CKD stage 3-5 . End stage renal disease represents a stage where accumulation of toxins ,fluid and electrolytes normally excreted by kidney leads to uremic syndrome .This constellation of symptoms and signs will cause mortality if not harmful toxins are taken out of body by renal replacement therapy in the form of either by renal transplantation or dialysis .

Formulas for calculating GLOMERULAR FILTRATION RATE[4]:

1. **MODIFICATION OF DIET IN RENAL DISEASE STUDY** which incorporates persons serum creatine and age

2. **Cockcroft Gault equation**

$$\text{Estimated creatinine clearance} = \frac{(140 - \text{age}) \times \text{body weight}}{72 \times \text{plasma creatinine}} = \text{ml/min}$$

Multiply 0.85 for women

Normal GFR is around 125ml/min per 1.73 m2

Pathophysiology of chronic kidney disease :

Consists of two types of mechanisms of damage :

1.First there will be start of mechanism specific to the cause of particular disease (abnormalities which affect kidney development genetically or integrity of kidney ,deposition of immunoglobulin and glomerulonephritis causing inflammation or tubular or interstitial diseases caused by certain types of toxins).

2.A collective and compensatory mechanisms of progressive hyperfiltration and hypertrophy of remaining functioning nephrons that happens in all kidney diseases leading to nephron loss whatever may be the etiology causing such events

Response to decrease in number of nephrons are mediated by vasoactive substances like growth factors and cytokines . Ultimately these short term adjusting mechanisms of hypertrophy and hyperfiltration become difficult as the intraglomerular hypertension and increased flow through remaining nephrons predisposes to loss of glomerular architecture associated with thickening and loss of existing nephrons . Elevated intrarenal action of renin angiotensin system is thought to contribute both to the initial adoptive increased filtration and to subsequent maladaptive hypertrophy and thickening of nephrons . Sclerosis develops due to action

of transforming growth factor beta. Because of this mechanism an acute short lasting damage to the kidney leads to progressive decrease in kidney function over many years and lead to the chronic kidney disease.

In chronic kidney disease there is progressive decline in the number of functioning nephrons and progressive decline in renal functioning . Up to some extent remaining nephrons compensate for the function and ultimately lead to decomponsated state leading to uremic syndrome .

Response of kidney to reduction in numbers of functioning nephrons :

Loss in the many functioning nephrons produces an increase in renal flow. Glomerular hyperfiltration which is the result of increased vasoconstriction in the post glomerular efferent arterioles compared to afferent arterioles increasing intraglomerular capillary pressure and filtration fraction .Persistent intraglomerular hypertension is associated with progressive nephron destruction . Hormonal and metabolic factors mediating hyperfiltration not fully understood. A number of vasoconstrictive and vasodilator substances have been implicated. One of the important factor is angiotensin 2. Angiotensin 2 causes vasoconstriction of efferent arterioles . Studies have shown that angiotensin converting enzyme 2 inhibitors will decrease intraglomerular capillary pressure and decrease proteinuria and slow the rate of nephron destruction .The vasoconstrictive agent endothelin has been implicated in hyperfiltration and

afferent vasodilatation has been attributed to local prostaglandins and release of endothelium derived growth factor that is nitric oxide .

Hyperfiltration may be mediated by resetting of kidney's intrinsic autoregulatory mechanism of glomerular filtration by a tubuloglomerular feedback system . Feedback originates from macula densa and modulates the renal blood flow and glomerular filtration .

Even with loss of functioning nephrons kidneys will maintain glomerular and tubular balance by which the remaining tubules adjust to increase in single nephron glomerular filtration with appropriate modification in the reabsorption or excretion of filtered water and solutes in order to maintain the homeostasis . Glomerular balance results from both increase in tubular size and from regulatory adjustments in tubular oncotic pressure or solute transport along the proximal tubule . Some studies indicate these alterations in tubule size and function may themselves be maladaptive and as a trade off predispose to further tubule injury .

Risk factors for CKD :

Throughout the world most common cause of CKD is diabetes mellitus .Other risk etiology for CKD are

- Hypertension and hypertensive nephropathy
- Glomerulonephritis

- Primary glomerular diseases with hypertension
- Vascular and ischemic renal disease

➢ polycystic kidney disease

➢ Other cystic and tubulointerstitial nephropathy

Identification of risk factors for CKD :

Predisposing Factors include

- Increasing age
- Increased blood pressure
- Poor blood glucose control and uncontrolled diabetes mellitus
- Autoimmune disease
- Family history of renal disease
- Previous episode of acute kidney injury
- Presence of proteinuria
- Presence of abnormal urinary sediment
- Structural abnormalities of urinary tract

Recently lot of developments have taken place in the field of genetics to find out specific loci for CKD . One among those is APOL1 gene which has been associated with CKD in west African ancestry

As CKD worsens GFR decreases . From third decade onwards annual decline in GFR from mean GFR 125 ml/min is **1ml/min /year per 1.73m2**. GFR is lower in women compared with men .

Measurement of albumin in urine helps in identification of nephron loss especially in patients with chronic glomerular disease .

Measurement of protein to creatinine ratio also helps in finding out renal disease Persistent of more than 17 mg of albumin per gram of creatinine in adult male and more than 25 mg of albumin per gram of creatinine in women usually signifies chronic renal damage .

Microalbuminuria is excretion of 30 to 300 mg of albumin per day is very useful indicator of presence of renal disease . Microalbuminuria is a marker of microvascular disease of the kidney .

Staging of CKD can be done according to GFR as stated above .

In Stage 1 & 2 even though there is decrease in GFR, are not associated with any symptoms but patient may present with symptoms related to etiology of CKD .For example if patient has autosomal dominant polycystic kidney disease he may present with complaints related to hypertension or a patient with nephritic syndrome may present with symptoms like generalized edema due to protein loss .If GFR further decreases patient

enters to stage 3 and then 4 then 5 . During these stages patient present with symptoms related to renal insuffiency .

Variety of manifestations due to renal insuffiency may develop .

Common during initial stages are easy fatigability, decreasing appetite ,with progressive malnutrition .

Later patient may develop imbalance in calcium ,phosphorous , Vit D metabolism , sodium , potassium, water and acid base imbalance . Eventually patient will land up in UREMIC syndrome .

Pathophysiology and biochemistry of Uremia:

Eventhough blood urea and serum creatinine are commonly used to find out excretory capacity of kidney there are many more waste products of metabolism are accumulated In body which are responsible for uremic syndrome

To name few they are

- Guanidine compounds
- Urate
- Hippurate
- Products of nucleic acid metabolism
- Polyamines

- Myoinositol
- Phenols
- Benzoates
- Indoles

Middle molecules with molecular mass between 50 to 500 Da are also retained and contributes to symtoms related to uremia and leads to morbidity and mortality .

Above lines show that blood urea and serum creatinine are easily measurable compounds but symptoms of uremia may be caused by variety of compounds

Functions of kidney like metabolic and endocrine functions deranged in CKD and contributes to morbidity and mortality .

Plasma levels of many hormones are altered in CKD as well as metabolism of protein sugar and fat metabolism

Parathormone ,FGF -23, insulin ,glucagon ,steroid hormone , testosterone and estrogen ,prolactin levels and metabolism of these hormones altered in CKD

Common causes of end stage renal disease[5] :

Disease	Proportion	Comments
Congenital and inherited	5%	Polycystic kidney Alports syndrome
Renovascular disease	5%	Atheromatous disease
Hypertension	5-20%	
Glomerular disease	10-20%	Ig A NEPHROPATHY
Interstitial disease	20-30%	Often drug induced
Systemic inflammatory disease	5-10%	Lupus nephritis , vasculitis
Diabetes mellitus	20-40%	
Unknown cause	5-20%	

Clinical manifestation of CKD :

Uremia causes dysfunction in almost all organs .Dialysis to some extent helps to decrease symptoms related to uremia but many conditions are not reversible with dialysis .

Fluid and electrolyte and acid base imbalance :

There will be retention of sodium and water in body in CKD. In normal person urine output matches intake of fluid . But in CKD patients there will be more reabsorption of water and sodium which leads to expansion of extracellular fluid content . This leads to hypertension in CKD patient .

Extracellular fluid expansion is isotonic as long as kidney capacity to excrete fluid and sodium is normal .Once capacity decreased there will be hyponatremia that is usually dilutional and will respond to water restriction . Diuretics may be needed to decrease volume overload . As renal function deteriorates further there will be more and more diuretics resistance requiring early initiation of dialysis

Potassium homeostasis :

Potassium homeostasis is deranged in CKD . It is not necessary that decrease in renal function causes increase in body potassium . It is because potassium excretion depends on aldosterone in distal nephron segments and potassium excretion through Gastrointestinal tract increased in CKD patients

Precipitating factors for hyperkalemia in CKD patients are

- Increased dietary potassium intake
- Protein catabolism especially in fasting state
- Hemolysis ,hemorrhage
- Transfusion of stored red blood cells
- Metabolic acidosis

Most important medication that causes increased potassium is ACE inhibitors , Angiotensin receptors blockers ,spiranolactones and other potassium sparing diuretics like amiloride ,triampterene

Some forms of CKD are associated with early disruption of potassium homeostasis out of proportion to decrease in GFR . In those category include mainly diabetic nephropathy , obstructive uropthy , sickle cell nephropathy .

Hypokalemia not so common in CKD .Its presence indicates severe dietary potassium lack , severe gastrointestinal potassium loss , excessive diuretic therapy

Metabolic acidosis:

Its is very common in advanced CKD .These patients cannot excrete adequate quantities of protons .Hyperkalemia increases the incidence of metabolic acidosis through decreased production of ammonium ions . Hyperkalemic , hyperchloremic metabolic acidosis is very common even in earlier stages of CKD . The latter finding is more common in patients with diabetic nephropathy and those with tubulointerstitial disease and obstructive uropathy

With decreasing renal function daily acid excretion decreases to less than 30 to 40 mmol per day. Anion gap metabolic acidosis is caused by retained organic acids . In most patients metabolic acidosis is mild and pH is rarely less than 7.35.It can be corrected with oral sodium bicarbonate supplementation . Alkali supplementation also slows down progression of CKD and is routinely prescribed for patients when bicarbonate concentration is less than 20-23 mmol/lit

Disorders of calcium and phosphate metabolism :

These occur in skeleton and vascular tissues Bony changes in CKD occur due to high bone turn over with increased parathormone level , and low bone turnover due to decreased paratharmone level

In CKD there will be **hyperparathyroidism** due to

- Retention of phosphate due to diseased kidney
- Decreased level of ionized calcium

These changes will start when GFR falls below 60ml/min

Fibroblast growth factor -23 is an important phosphatonin that promote phosphate excretion .It is elevated early in CKD .It will be elevated early in CKD which will protect against development of hyperparathyroidism .

Hyperparathyroidism stimulates bone turnover and leads to osteitis fibrosa cystica . PTH is considered as important uremic toxin and high level of which is associated with muscle weakness ,fibrosis of cardiac muscle , nonspecific symptoms

Low turnover of bone lead to adynamic bone disease and osteomalacia which will lead to increased incidence of fractures and increased vascular and cardiac calcification .

In CKD patients calcification in media of coronary artery and in heart valves is of greater proportion compared to non CKD patients

Calcific uremic arteriolopathy that is almost exclusively occurs in patients with CKD . Pathology involves vessel obstruction and enormous vessel wall and soft tissue calcium deposition . Previously it was associated with hyperparathyroidism . But recently it is found even in the absence of hyperparathyroidism .Warfarin used in dialysis is found to be risk factor for development of calciphylaxis

Cardiovascular abnormalities :

Cardiovascular abnormalities are main causes of mobidity and mortality among patients with CKD.

Risk of cardiovascular diseases increases by 10 to 200 fold in CKD patients depending on stage of CKD . In stage 5 CKD almost all patients have some form of cardiovascular abnormality . So management of CKD in earlier stages should aim at preventing development and progression of cardiovascular complications.

Important among cardiovascular diseases in CKD patients is ischemic vascular disease which include

- Blockage of coronary arteries due to atheroma
- Diseases of vessels supplying brain
- Vessels of periphery

In CKD there will be additional risk factors that add to cardiovascular diseases like

- Anemia
- Hyperparathyroidism
- Hyperphosphatemia
- Sleep apnoea
- Generalized inflammation

This along with traditional risk factors like hypertension ,hypervolemia , dyslipidemia , sympathetic overactivity lead to increased incidence of vascular diseases .In CKD there will be additional increased circulating

acute phase reactants like cytokines and C reactive protein with fall in albumin and fetuin

This inflammatory state accelerates vascular occlusive disease and decreased fetuin causes rapid vascular calcification .

In CKD there will be left ventricular hypertrophy and microvascular disease that leads to myocardial ischemia .These things causes myocardial infarction and congestive cardiac failure .

Cardiac troponins are usually elevated in patients with CKD without evidence of myocardial ischemia . This elevation causes confusion in diagnosing myocardial infarction unless serial measurements are done .

Heart failure :

In CKD there is abnormal cardiac hypertrophy ,myocardial ischemia and cardiomyopathy with salt and water retention that leads to heart failure and pulmonary edema

It may due to systolic or diastolic dysfuncfion .

In uremic state there is increased permeability of capillary membranes that lead to pulmonary edema even in the absence of increased pulmonary capillary pressure .

Hypertension and left ventricular hypertrophy :

Hypertension is very common CKD. Presence of hypertension leads to rapid deterioration of kidney function, early left ventricular hypertrophy

Presence of anemia and creation of arteriovenous fistula leads to high output failure . Chronic extracellular fluid overload also increases blood pressure. So diuretics , salt restriction ,dialysis to remove excess fluid will decrease blood pressure and leads to better cardiac functioning .

Pericardial disease:

Pericarditis is common in uremia which leads to chest pain. It may also associated with pericardial effusion leading to cardiac tamponade. Pericarditis is observed in advanced uremia. More common in underdialyzed , nonadherent patients than in those starting dialysis .

Hematological abnormality in CKD [6] :

Anemia is most common abnormality seen .It is most commonly normocytic , normochromic . It appears as early as in stage 3 and almost common at stage 4 & 5 .

Anemia in CKD caused by :

- Decreased erythropoietin production
- Diminished red blood cell survival

- Bleeding diathesis
- Iron deficiency
- Hyperparathyroidism and bone marrow fibrosis
- Chronic inflammation
- Folate and Vit B 12 deficiency
- Hemoglobinopathy
- Comorbid conditions

Abnormal hemostasis :

Patients with CKD especially in terminal stages have increased tendency to bruising, bleeding from surgical sites ,menorrhagia and spontaneous bleeding. They have increased bleeding time,reduced functioning of platelet factor 3, platelet aggregation defect, adhesiveness.and defect in prothrombin utilization . CKD patients are more prone for thromboembolism especially those with nephrotic range proteinuria .

Neuromuscular abnormality[7]:

All three namely central nervous system , peripheral nervous system, autonomic nervous system are involved in CKD , as well as muscle . Nitrogenous waste products , middle molecules ,are responsible for this . As early as stage 3 CKD neurological disease may appear .

Neuromuscular irritability , hiccups ,cramps ,fasciculations or twitching may develop

In advanced cases asterixis , myoclonus ,seizures , coma may develop.

Peripheral neuropathy develops at the stage of 4.

Initially sensory more than motor , lower limb more than upper limb ,distal more than proximal involved .

Restless leg syndrome is peculiar abnormality seen in CKD patient .

Presence of neuropathy in patients with CKD is an indication for renal replacement therapy

Gastrointestinal abnormality[8]:

Uremic fetor is a urine like odour of breath . It occurs due to breakdown of urea to ammonia in saliva and associated with metallic taste.

Other abnormalities like gastritis , peptic ulcer disease, mucosal ulceration may develop .Patient may develop constipation that is aggravated by administration of iron and calcium supplementation. Decreased appetite, nausea and vomiting may develop .

Endocrine and metabolic disturbances[9] :

Glucose metabolism is impaired in patients with CKD .But fasting blood glucose will remain normal in patients with CKD .

Insulin is degraded by kidney .So in kidney dysfunction insulin level will be elevated .

In women estrogen level is low .There may develop abnormalities related to menstruation and pregnancy .They may develop spontaneous miscarriage especially when GFR has dropped below 40ml/min .

Men with CKD have decreased testosterone levels . They may suffer from sexual dysfunction and decreased sperm count .

Dermatological abnormality:

Common with CKD especially in late stages.

Pruritis is the most common condition in uremic state .

Patients are hyperpigmented due to deposition of pigmented retained metabolites like urochrome .

Nephrogenic fibrosing dermopathy ,is a condition in which there is progressive subcutaneous induration especially in arms and legs .It is most common in patients with CKD with exposure to Magnetic resonance scan(MRI)with gadolinium contrast .So in patient with CKD who undergo

MRI Scan with contrast it is recommended to remove contrast material through hemodialysis .

Diagnosis of CKD :

Initial history and physical examination :

Signs and symptoms during initial stages are often minimal . When stage reaches 3 or 4 patient becomes symptomatic . Initial symptoms are mild and often subtle .When the patient comes to know that they are having CKD its often surprising . History of hypertension , diabetes mellitus and chronic drug intake may point towards the cause of CKD . Chronic drug intake especially NSAIDS other anti-inflammatory agents , gold penicillamine, antimicrobials , chemotherapeutic agents , antiretroviral agents ,proton pump inhibitors , lithium and other drug history should be investigated as a part of diagnostic questionare .

Physical examination should be done to find out target organ damage due to hypertension arising from CKD . Fundus examination to find out retinopathy , cardiac examination to find out left ventricular failure should be carried out

Laboratory evaluation :

Tests should focus on finding clues to the cause of CKD , aggravating factors , degree of renal damage and its consequences . Serum and urine protein electrophoresis should be obtained in all the patients who are above 35 years of age to rule out multiple myeloma especially if there is anemia and increased serum calcium level in the face of renal insufficiency

In presence of glomerulonephritis autoimmune diseases such as lupus nephritis and underlying etiologies such as hepatitis B and hepatitis C and HIV should be investigated . Serial measurements of renal parameters like blood urea and serum creatinine to ensure that renal damage is chronic and not an acute deterioration and reversible . Serum concentrations of calcium, phosphorus , vitamin D , and Parathormones should be measured as a part of evaluation of metabolic bone disease . Hemoglobin concentration , iron , Vit B 12 and folate should be evaluated . 24 hour urine protein must be evaluated which will help to find out >300 mg protein excretion to suggest therapy with ACE inhibitors or ARBS .

Imaging studies :

The most important and useful investigation is renal ultrasonography which will help to find out presence or absence of two kidneys,whether they are symmetric , size of both the kidneys, to rule out renal mass or

evidence for renal obstruction . As CKD is a chronic process there will be contraction of kidney . Presence of bilaterally small kidneys support the diagnosis of CKD of long standing duration with an irreversible component of scarring .If the kidney size is normal it indicates that the process is acute or subacute . The exception to this are diabetic kidney disease which often show normal or increased kidney size and amyloidosis and HIV nephropathy where kidney sizes are normal in the pace of CKD . Polycystic kidney disease show often enlarged kidney with multiple cyst. A discrepancy of more than 1 cm in kidney length suggest either a unilateral developmental defect or a disease process or renovascular disease with arterial insufficiency affecting one kidney more than other . Diagnosis of renovascular disease can be done by Doppler ultrasonography, nuclear medicine studies , CT or MRI .If there is suspicion of reflux nephropathy a voiding cystourethrogram should be done

Renal biopsy :

Usually done under the guidance of ultrasongraphy is the favoured approach . Biopsy is not indicated in bilateral small kidneys because

1. If it is technically difficult to perform and risk of major bleeding and other adverse consequences
2. There is so much scarring that underlying disease may not be apparent

3. Window of opportunity for specific therapy has passed

Other contraindication for renal biopsy are

1. Uncontrolled hypertension
2. Active urinary tract infection
3. Bleeding diathesis
4. Severe obesity

A short run of hemodialysis before renal biopsy may be useful to normalize bleeding time in those who have increased bleading time

Establishing diagnosis of chronicity of disease :

It is important to differentiate between acute kidney failure from chronic renal failure because acute renal failure is often reversible if appropriate measures are taken and needs only short time management until kidneys recover its functions. If previous values are known , recent elevation points to more acute process for renal dysfunction . In contrast if elevated serum concentration in the past suggests that renal disease represents a progression of chronic process . Even if the disease is chronic an acute insult in the form of dehydration , or toxin exposure or sepsis might have precipitated a sudden worsening of kidney function

Some other parameters suggest a chronicity of desease . The presence of metabolic bone disease with hyperphosphatemia , hypocalcimia , elevated PTH and bone alkaline phoshatase suggests chronicity. Normocytic normochromic anemia suggest that the process is ongoing for long duration.

The presence of bilateral reduced kidney size (< 8.5 cm in all but the smallest adults) suggest chronicity .

Diagnosis of etiology of CKD :

Renal biopsy is may be helpful in diagnosis of CKD but it is not always necessary . If a patient is suffering from diabetes for long duration and he has nephrotic range proteinuria its very obvious that cause of CKD is diabetic nephropathy . It is also very clear that a person suffering from hypertension that ischemic nephropathy the cause of CKD . If diagnosis is not obvious renal biopsy may be undertaken .

Criteria for referral of chronic kidney disease patients to nephrologist :

- Age of patient < 40 years
- Stage 4 CKD or worse
- Rapid deterioration in renal function
- Significant proteinuria (PCR > 100 mg /mmol or ACR > 70 mg /mmol
- Significant hematuria

Treatment of CKD :

Depends on stage of CKD

STAGE	EXPLANATION	GFR ml/min per 1.73m2	Treatment
1	Kidney damage with normal or increased GFR	>90	Diagnosis and treatment of comorbid condition, slowing the progression , cardiovascular risk reduction
2	Kidney damage with mild decrease in GFR	60-89	ESTIMATING PROGRESSION
3	Moderate decrease in GFR	30-59	Evaluating and treating the complication
4	Severe decrease in GFR	15-29	Preparation for kidney replacement therapy
5	Kidney failure	< 15 or on dialysis	Kidney replacement

Slowing the progression of the CKD :

Reducing intraglomerular pressure and proteinuria :

Control of systemic and glomerular hypertension are important in slowing down the progression of chronic kidney disease .So antihypertensive therapy aimed at reduction of intraglomerular pressure .Proteinuria is increased by systemic hypertension due to increased flux across glomerular capillaries. Antihypertensive medication decreases the proteinuria thereby decreases the decline in GFR further .So target blood pressure of 125/75 has been kept for CKD patient to reduce proteinuria and progression of CKD .

ACE inhibitors and Angiotensin receptor blockers are found useful in progression of CKD . They cause decrease in intraglomerular pressure by inhibiting angiotensin induced constriction of efferent arterioles

They are also found to decrease proteinuria and progression of CKD .

Calcium Channel blockers like verapamil and diltiazem are also found useful

Slowing progression of diabetic renal disease:

Blood glucose control:

Tight glycemic control decreases the chances of renal disease

Control of blood pressure and proteinuria :

Hypertension is seen in majority of patients with diabetes mellitus. This finding correlates with presence of albuminuria and it is one of the important predictors of cardiovascular events and nephropathy. Antihypertensive treatment reduces the albumin excretion even in normotensive patients

Patients should be planned for renal replacement therapy either in the form of dialysis or transplantation once end stage renal disease establishes .

Dialysis in patients with chronic kidney disease may be may be indicated in

1. Presence of uremic symptoms such as uremic gastritis , uremic pericarditis
2. Electrolyte disturbance such hyperkalemia unresponsive to conservative therapy
3. Persistent extracellular volume expansion despite adequate diuretic therapy
4. acidosis refractory to medical therapy
5. bleeding diathesis

6. creatinine clearance or estimated glomerular filtration rate below 10 ml/min per 1.73 m2

In end stage renal disease the treatment options may be

Hemodialysis

Peritoneal dialysis as continuous ambulatory peritoneal dialysis or continuous cyclic peritoneal dialysis

Transplantation

Hemodialysis :

It relies on principle that of solute diffusion across a semipermeable membrane . Movement of metabolic waste products takes place down the concentration gradient from the circulation into dialysate .The rate of diffusive transport increases in response to several factors like magnitude of concentration gradient , membrane surface area and the mass transfer coefficient of the membrane . According to law of diffusion , the greater the size of molecule slower its rate of transfer across the membrane . Smaller molecules like urea (60 Da) undergoes substantial clearance , but a larger molecules like creatinine(113Da) undergoes less clearance . In addition to diffusive clearance there take also place ultrafiltration of waste products from the circulation into the dialysate .

Complication of hemodialysis ：

1. Hypotension is the most common acute complication of hemodialysis . Factors affecting development of hypotension are rate of ultrafiltration, impaired vasoactive or autonomic response , osmolar shift ,excessive use of antihypertensive agents ,reduced cardiac reserve.

Patient with arteriovenous fistula may develop high output cardiac failure through shunting of blood through dialysis access . Acetate used in dialysate because of its vasodilatory and cardiosuppressive effect may aggravate hypotension .

2.Muscle cramps are also common during dialysis .Etiology for this is obsure . Changes in muscle perfusion due to excessive aggressive volume removal and use of sodium containing dialysate may be the cause .

3 .Anaphylactoid reaction commonly with first time use especially with bioincompatible cellulose containing membrane . With use of cuprophane membrane this dialyser reaction has become less common .

4. Progressive loss of remaining nephrons is expected in patients on chronic hemodilaysis due to decreased work of remaining nephrons and atrophy

5. Cardiac arrhythmias

6. Air embolism

Between treatment patients may develop pulmonary edema and systemic sepsis

Peritoneal dialysis :

In peritoneal dialysis , 1.5 to 3 litres of dextrose containing solution is infused into peritoneal cavity and allowed to dwell for a set period of time around 2-4 hours .Toxic materials are removed through a combination of convective clearance generated through ultrafiltration and diffuse clearance across a concentration gradient .The clearance of solutes and water depends on movement of water and solutes into the peritoneal cavity versus absorption from peritoneal cavity .Rate of diffusion diminishes with the time as equilibrium is reached between plasma and dialysate .Rate of solute transport varies from person to person and altered by factors such as peritonitis , drugs , physical factors like position of patient and exercise .

Types of peritoneal dialysis :

1. Continuous ambulatory peritoneal dialysis
2. Continuous cyclic peritoneal dialysis

Complicatios of peritoneal dialysis :

Peritonitis is most common complication in patients with peritoneal dialysis. It can be suspected by drainage turbid dialyste or finding more than 100

leukocyte withmore than 50% polymorpholeukocytes. Staphylococcus is the most common organism indiacating travel of organism from skin due to breach in sterile technique in access to peritoneal cavity . It is usually managed with intraperitoneal or intravenous antibiotics .

Patients on peritoneal dialysis are prone to number of metabolic complications . Due to high dextrose in dialysate patients are prone to hyperglycemia especially patients with diabetes mellitus who develop insulin resistance . Due to loss of albumin in dialyate fluid patient develop hypoproteinemia and its complications .

Sclerosing peritonitis may develop due to infectin and inflammation .

Transplantation :

If HLA matched donors are available transplantion of kidney can be undertaken in patient with CKD in the ESRD .It may be an orthotopic or heterotopic kidney transplantation . Nowadays heterotopic kidney transplantation are performed frequently .

Contraindication for renal transplantation are ;

Absolute contraindication :

Active malignancy ; A period of atleast two years of complete remission is recommended for most tumour

Active vasculitis or recent anti GBM diseases

Severe heart disease

Severe occlusive aorto -iliac vascular disease

Relative contraindication :

Age , transplantation not done routinely for child < 1 years or > 75 years

High risk of disease recurrence in transplanted kidney

Diseases of the lower urinary tract

Significant comorbidity

Complication of transplantation :

Graft failure

Drug toxicity due to use of immunosuppressents

Graft versus host disease

Opportunistic infection particularly with cytomegalovirus

Hypercalcemia

Hypertension due to residual kidney diseases

Chronic hepatitis

Causes of death in CKD :

Cardiovascular disease constitutes major cause of death in patients with CKD . Cardiovascular events are more common in patients with hemodialysis than with post transplantation patients. Increased cardiovascular morbidity may be due to diabetes mellitus ,hypertension , atherosclerosis , dyslipidemia ,anemia , dystrophic vascular calcification , hyperhomocysteinemia .Patients are highly prone for myocardial infarction , cerebrovascular accidents .

Other causes of death are severe anemia with heart failure . infections , dialysis related complications , comorbid condition which has caused the CKD

Homocysteine :

In 1932 Botz and Vizwand initially described homocysteine .But its link with human disease was described only later in 1962

Inborn errors of homocysteine leads to very high homocysteine level . Physician Dr McCully demonstrated that early onset of atherosclerosis in patients with increased homocysteine even in children . He stated that even mild elevation of homocysteine can cause atherosclerosis [10].But people rejected his hypothesis

Homocysteine formed during metabolism of methionine .It is a thiol containing intermediate aminoacid. Average plasma total homocysteine for an healthy human being is less than 15micromol/lit. . Allon Freidman describes in journal of kidney and homocysteine metabolism that in the era of fortification with folic acid the amount of normal homocysteine in plasma is 6 to 12micromol/lit .

Mild hyperhomocysteinemia at level of 12 to 30 micromol/lit

Intermediate hyperhomocysteinemia at level of 31 to 100 micromol/lit

Severe hyperhomocysteinemia as more than 100 micromol/lit

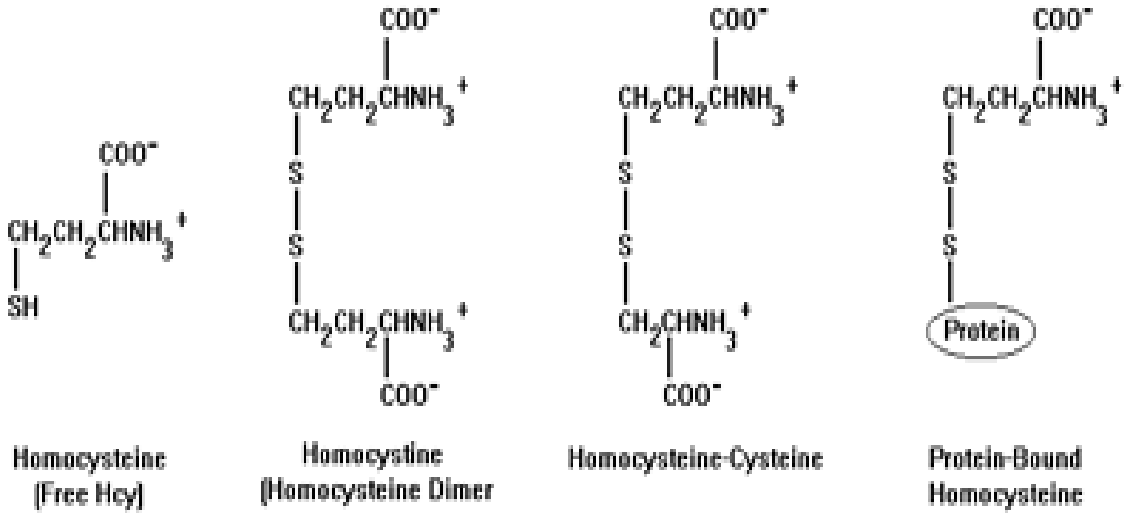
In normal persons 75% of total homocysteine are bound through a disulphide bond to proteins mainly to albumin .

Remaining 25% remains in the free form . Free form exists in almost oxidized form as disulphide linked heterodimers(homocysteine-cysteine) or as homodimers (homocysteine –homocysteine) [11.]Free homocysteine is unstable and its level is difficult to measure . Free homocysteine is the only only one which freely filters through kidney at glomerulus . fHcy/bHcy fraction varies among different species .

Homocysteine is produced in all the cells due to normal methylation process .

Intracellular homocysteine level increases either because of increased homocysteine production or due to inhibition of intracellular metabolism

Homocysteine is regularly synthesized and exported out of cells there must be some mechanism to keep plasma level within normal limit .



Types of homocysteine

Homocysteine is synthesized and metabolized by three different process [11]

1. demethylation process
2. transmethylation process
3. Transulfuration process

Hcy is synthesized inside the body from essential aminoacid methionine and is not obtained from diet

Demethylation

In this process methionine is converted into intermediate metabolites like S-adenosyl methionine and S-adenosyl homocysteine.

Transmethylation

Remethylation of homocysteine to methionine occurs in this process.

By using Vit B 12 and 5- mehtyltetrahydrofolate as a substrate homocysteine is converted back into methionine .

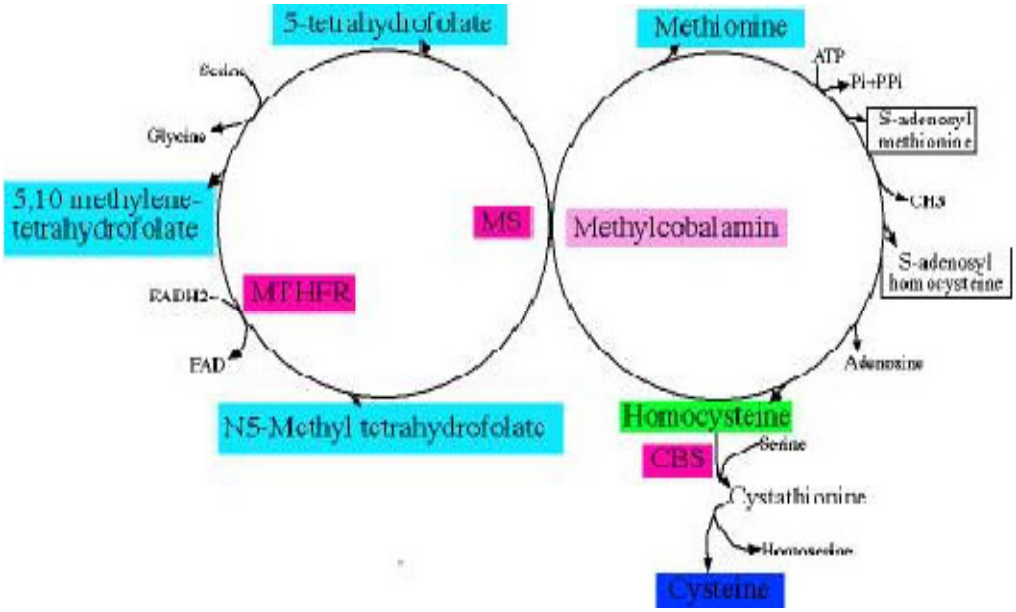
Betain homocysteine methyl trasferase remethylates homocysteine to methionine in liver and kidney mainly in an alternate reaction .

Transsulfuration

In this process, cysteine formed from homocysteine in an irreversible reaction .

Reaction is catalysed by cystathionine **B-**synthase , a pyridoxal-5 phosphate /Vit B6 dependent enzyme to form cystathionine. Cystathionine is hydrolysed to form cysteine ,ammonium and alpha ketoglutarate

Folate or Vit B12 deficiencies causes increased homocysteine level by impairing homocysteine remethylation . Vit B6 deficiency causes defective transulfuration and increased homocysteine level .



METABOLISM OF HOMOCYSTEINE

Measurement and Classification of Homocysteine Levels

The normal level of plasma homocysteine is 5-15 µmoles/lit .Level in the plasma greater than 15 µmoles/L is considered as hyperhomocysteinemia. Hyperhomocystenemia classified by American Heart Association into [12]

Moderate : homocysteine level 15-30 µmoles/L

Intermediate : 30 - 100 µmoles/L

Severe : >100 µmoles/L

Measurement of plasma homocyestine:

A blood sample, which preferably in a fasting patient, is the most commonly taken test to assess homocysteine status. Careful handling of sample is most important to correct estimation of plasma homocysteine .

Plasma should be measured for homocysteine early after collection or blood kept at 4 degree celcius . If not done like this homocysteine level increases due to hemolysis . 3-deaza-adenosine or fluoride can be used for prompt stabilization of plasma homocysteine

Methods of estimation of plasma homocysteine [12]

1. ELISA
2. High performance liquid chromatography
3. Fluorescence polaraization immunoassay
4. Mass spectrometry

Classification of hyperhomocystinemia

Hyperhomocysteinemia classified into [13]

1. Primary
2. Secondary

1. Primary hyperhomocysteinemia

Due to defects in enzyme which are involved in homocysteine metabolism

a. Cystathionine beta synthase (CBS) deficiency:

The most frequent cause of hyperhomocystinemia due to genetic deficiency of enzyme involved in homocysteine metabolism . It is autosomal recessive disease .

It is characterized by premature atherosclerosis , dislocation of lens , skeletal abnormalities, mental retardation , homocystinuria..Affected persons will be having homocysteine level between 20-40umol/litre.Its incidence is around 1in 3 lakh

b. 5,10 methylene tetrahydrofolate reductase (MTHFR) deficiency

A mutation invoving the enzyme MTHFR is associated with hyperhomocysteinemia in persons with low folate level

c. Methylene tetrahydrofolate homocysteine methyl transferase deficiency[14]

2. Secondary hyperhomocysteinemia:

a. Physiological

- Male sex
- Increasing age
- Menopause

b. Lifestyle factors –

- Excessive Coffee consumption
- Tobacco use

c. Vitamin deficiency –

Vit B12 (cobalamine) ,Folic acid, Pyridoxine (Vit B6)

d. Systemic disorders

(i) Severe hepatic impairment

(ii) Pernicious anemia

(iii) Psoriasis

(iv) Renal impairment

(v) Systemic lupus erythematosus)

(vi) Hypothyroidism

(vii) Organ transplantation

(viii) Anorexia nervosa

(ix) Malignancies of breast and ovary

e. Drugs

1)) Folate antagonists – Carbamazepine, Phenytoin

2) cholestyramine ,colestipol ,metformin (affect folate and cobalamin absorption

3) L-dopa

4) Vit B6 antagonists (theophylline, oestrogen containing OCP ,niacin)

5) Androgens

6) Nitrous oxide (inactivates methionine synthesis)

7) cyclosporins , fibric acid derivatives (Causes renal dysfunction)

Determinants of plasma homocysteine level[15] :

Variable		Fasting plasma homocyteine level
Sex	Male	10.3
	Female	8.8
Age(years)		
	<45	8.8
	45-54	9.2
	54-64	9.8
	>65	10.4
Serum creatinine(mol/lit)		
	<79	8.7
	79-87	9.3
	87-96	9.3
	96-106	9.7
	>106	10.5
Alcohol intake(g/d)		
	0.1-4.9	9.3
	5.1-4.9	9.4
	>15	10.0
Caffeine intake(mg/d)		
	<88	8.9
	>420	9.9
Current cigarette smokers		
	0	9.3

	1-15	9.9
	16-25	10.1
	>26	11
Body mass index(kg/m2)		
	<23.2	9.4
	>30.6	9.9

Main determining factors of plasma homocysteine are vit B12 ,folic acid , vit B6 which are present in the food . A 0.5 to 5 mg of folic acid daily reduces the plasma homocysteine level by 25% . Adding vitamin B12 to folate further reduces the homocysteine levl by around 7% [16].

Functioning of kidney is an important determining factor of maintenance of normal homocysteine level because of its intrarenal homocysteine metabolism and excretion of homocysteine .

Coffee intake and smoking showed positive association with homocystiene.

Moderate alcohol lowers while chronic alcoholics have increased plasma homocysteine level.

Effects of Homocysteine:

Homocysteine produces atherosclerosis, favours intravascular thrombus formation and subsequent embolisation and damage to the vascular endothelium . It affects endothelial surface, plasma lipoproteins , vascular

smooth muscle cells , platelets, coagulation factors, blood lipids and connective tissues.

Homocysteine stimulates& increases DNA synthesis, cyclin activity, vascular smooth muscule proliferation . It causes decrease in activity of NA^+ - K^+ - ATPase and causes hemolysis of red blood cells .and increases platelet adhesion and aggregation

Homocysteine may facilitate increased binding of fibrin and lipoprotein (a). In high concentration it causes factor 5 activation , protein C inactivation, cofactor activity of thrombomodulin inactivation , suppression of thrombomodulin.

Hyperhomocysteinemia and atherosclerosis[17]:

Homocysteine preidisposes to atherosclerosis and thrombosis by causing [18]

1. Endothelial injury
2. Platelet activation
3. Smooth muscle proliferation
4. Oxidative modification of low density lipoprotein
5. Endothelial – leukocyte interaction

Endothelial injury :

Endothelium is a comprising vascular inner layer will maintain hemostasis in vessesls and support proper blood and oxygen supply to the tissues .

According to response to injury hypothesis the primary event in the atherosclerosis is damage to the endothelium and malfunctioning of which halts many normal characters of endothelium resulting in leukocytes and platelet adhesion ,thrombus formation proliferation of smooth muscles , spasm of vessel ,lipid collection ,and finally atherosclerosis

Homocysteine concentration between 1-10 umol/lit was found to cause damage to vascular endothelial cells depending on dosage in human umbilical vein . Hydrogen peroxide generated by the copper catalysed autooxidation of homocysteine in which copper acts as catalyst was thought to cause vascular endothelial damage by the demonstration of reduction in toxicity by addition of catalse . There was high strand breakage and decreased level of nicotinamide adenine deaminase level in endothelial cells which have been incubated in high concentration of homocysteine .This shows homocysteine causes induction of DNA breakage ,also prevents DNA production in human umbilical vein endothelial cell in a manner which depends on concentration of homocysteine . These effects on DNA causes not only damage to endothelial cells also impairs ability to regenerate following injury and decreased synthesis of enzymes involved in transmethylation and transulfuration . This shows attack on DNA is an important mechanism of injury on endothelial cells by mediated by homocysteine

Endothelial dysfunction :

Mechanism of endothelial dysfunction may be due to decreased nitric oxide or other mechanisms . Role of those substances involved and mechanism causing endothelial dysfunction are narrated below.

1.Nitric oxide :

Nitric oxide , endothelial derived regulates vascular tone ,prevents platelet activation ,adhesion ,aggregation ,limits smooth muscle proliferation ,and modulates leukocyte and endothelial interaction .

Homocysteine forms S- nitrosohomocysteine by reacting with nitric oxide , which acts in a similar way to nitric oxide .It causes vasodilation ,inhibits platelets aggregation .This is one of the protective mechanism against homocysteine mediated injury . It has been demonstrated that patient under oxidant stress shows that there is impairement of vascular nitric oxide activity by homocysteine .

Studies conducted both inside and outside the body shows that homocysteine causes decreased synthesis of nitric oxide by endothelial cells and they cause breakdown of nitric oxide structure by oxidizing it ,there by homocysteine decreases nitric oxide bioavailability . This is one of the most important and powerful mechanism by which homocysteine in increased concentration facilitates atherosclerosis and thrombus formation .

2**. Gluthathione peroxidase:**

Hydrogen peroxide and peroxides of lipid are reduced to respective alcohols by Glutathione peroxidase , a substance derived from endothelial cells and it protects against oxidant stress induced damage to cells .Activity of glutathione peroxidase was found lower in hyperhomocysteinemia .There was a similar reduction in glutathione peroxidase mRNA levels in those patients . Homocysteine causes inhibition of glutathione peroxidase activity in vitro . It is the only one among thiols which decreses the glutathione peroxidase activity .It is also helping to explain its greater toxicity by generating free radicals .

Disturbance of blood hemostasis :

Thrombosis is prevented on the vascular endothelial surface due to presence of surface glycosaminoglycan- antithrombin III and thrombomodulin –protein C anticoagulation pathway, synthesis of plasminogen activators and inhibition of platelet activators , clotting factors which are synthesized by endothelium balances between propensity towards clot formation and and in maintaining liquidity of blood there by helping to either thrombus formation or preventing thrombosis under appropriate condition .

This disturbance in hemostasis caused by hyperhomocysteinemia may be by

1.Stimulation of procoagulant factors ;

In bovine studies it has been shown that after treatment with homocysteine bovine endothelial cells exhibit more factor V activity. Mechanism of action is induction of proteases endothelial cell activator of factor V and gives an explanation for thrombosis by homocysteine in the absence of thrombin .

2.Prevention of anticoagulation mechanisms:

Endothelial cell of pigs incubated in homocysteine have shown reduced expression of heparin like glycosaminoglyacans . Hyperhomocysteinemia patients also exhibit decreased functional antithrombin III, as shown in small number of patients .Bovine endothelial study has shown competitive inhibition of thrombin to thrombomodulin activation by homocysteine . Some research activists also telling that that homocysteine is responsible for inactivation of both thrombomodulin and prorein C either directly or indirectly . Thrombomodulin expression on the cell surface decreased by homocyteine by disturbing the transit along the secretory pathway . Deliberate infusion of diet containing excessive homocysteine in monkeys have caused thoracic aorta to contain 35% lower thrombomodulin dependent protein C compared to normal diet infusion monkeys.

3. Fibrinolysis impairement :

Endothelial cells incubated with homocysteine was found to show less amount of both tissue plasminogen activator activity and membrane binding sites . Lipoprotein (a) ,a atherogenic lipoprotein reduces the fibrinolysis . Homocysteine facilitates lipoprotein (a) attachment to fibrin thereby decreasing the activation of plasminogen .

4.Alteration of platelet and endothelial interaction :

Activation of platelets causes thrombus formation within the vascular compartment and proliferation of smooth muscles . So it plays an important role in atherothrombosis. EctoADPases are susbstances which break down ADP and hence prevents platelets activation and aggregation . Homocysteine in high concentration prevent this ADPases and hence facilitates platelet activation and aggregation. Prolonged exposure of endothelial cell to homocysteine causes prevention of platelet aggregation which was originally causesd by nitric oxide . Studies have shown that acute elevation of homocysteine found to promote platelet aggregation and which was prevented by co-administration of antioxidant vitamins like vitamin E and C . It suggest that stress caused by oxidative forces can alter functioning of platelets .

Effects directly on platelets :

Increased homocysteine concentration causes increased release of vasogenic and thrombogenic agent called thromboxane , from plasma which are highly concentrated with platelets .

Mcdonald et al, demonstrated exaggerated adhesion of platelets in patients with homocysteinuria ,but there is no experimental evidence to show such kind of influence of homocysteine on platelet adhesion and aggregation .

Proliferation of vessel wall smooth muscle :

Proliferation of vessel wall smooth muscle is an important aspect of atherogenesis. This process is stimulated either by homocysteine directly or because of growth promoting effect of growth factors released by endothelial cells and platelets, which are released due to endothelial and platelet damage caused by excessive homocysteine .Homocysteine also found to cause decrease in deoxy nucleotide adenosine (DNA) production in endothelial cells of umbilical veins .As a whole homocysteine causes less damage to the smooth muscle cells than to endothelial cells . This appears to contribute to the mechanism in development of atheroma formation .

Oxidative proliferation of low density lipoprotein :

Modified low density lipoprotein due to oxidation are found to have an vital role in development of cholesterol induced atheroma formation and thickening of vessels .Products of lipid peroxidation which was indicated

by amount of one such product called thiobarbuturic acid was increased upon incubation with homocysteine in umbilical vein endothelial cells . At present there is only some proof to show that at high concentration of homocysteine increases the modification of low density lipoprotein due to oxidation .

Interaction between leukocytes and endothelial cell :

Adherence of white blood cells to endothelial cells is an vital process in atherogenesis . Human umbilical vein endothelial cells pretreated with homocysteine showed increased adhesion and transendothelial migration of neutrophils . Mechanism of increased interaction was supposed to be due to neutrophilic docking protein complex CD 11b /CD 18 and endothelial expression of monocyte chemoattractant protein -1 .

Complications of hyperhomocystinemia

1. Cerebrovascular disease
2. Ischemic heart disease
3. Hypertension
4. Peripheral vascular disease
5. Venous thromboembolism

Association between hyperhomocysteinemia, peripheral vascular disease and stroke.

Homocysteine level is increased in patients with cerebrovascular accident and diseases affecting vessels of periphery . As concerned with cerebrovascular disease, 11 clinical studies aimed at association between homocysteine levels and cerebrovascular disease. In nine studies, there was significant relationship while two prospective studies lacked evidence for an association. Clark et al, reported that measurement of homocysteine after methionine loading and subsequent measurement of homocysteine in men with early vascular disease before ageing revealed that hyperhomocysteinemia was observed in 28% of patients with peripheral vascular disease and 30% of coronary artery disease . 42% of patients suffering from diseases of vessels of brain [19]

Hyperhomocysteinemia and thrombosis of veins :

Increased homocysteine is an important independent risk factor for thrombosis of venous system and subsequent embolisation .

Martin Den Haijer et al.[20] reported that plasma homocysteine concentration in plasma of patients with history of repeated thrombosis of venous system was very high both in fasting state as well as after loading the patients with precussor substances like methionine as in the form of methionine loading test . The combination of increased homocysteine concentration and

mutated factor V further increased the relatve risk of venous thromboembolism upto 3.64 times .

Homocysteine and diabetes mellitus[21]

Elevated homocysteine levels were found in patients with type 2 diabetes mellitus who had macrovascular disease .

Hoogeveen et al. have demonstrated if we compare subjects with normal / impaired glucose tolerance hyperhomocysteinemia is a stronger risk factor for cardiovascular diseases in type 2 diabetes mellitus (1.6 fold)

Lakshman et al, reported that higher levels of homocysteine was found in patients with diabetic nephropathy (with microalbuminurea) , early type 1 diabetes mellitus ,patients with autonomic neuropathy and patients with long standing diabetes with poor glycemic control . They also reported that higher serum concentration of homocysteine found in hypertensive type 2 diabetes mellitus and diabetes with microalbuminurea but it was not statistically significant . They found significant association with homocysteine and diabetic retinopathy They reported a positive correlation between H bA1c and serum triglyceride with homocysteine levels in both the diabetic groups . They conclude by telling that higher homocysteine was found in diabetes patient with both microvascular and macrovscular complication and it was highly correlated with HbA1c and serum triglyceride levels which are indicators of poor diabetic control .They say that hyperhomocystenemia could serve as a another parameter indicating of

poor diabetic control and development of complication .So homocysteine level in diabetes needs to be monitored and if found elevated appropriate therapy to decrease is needed .

Homocysteine and hypertension[22]

Bortolotto LA et al have shown that hypertensive patients with high levels of homocysteine are associated with increased arterial stiffness.

Kim Sutton et al, in their article showed that homocysteine is independently and strongly associated with isolated systolic hypertension (ISH).According to them elevated blood pressure in older individual may be a cause of isolated systolic hypertension . Stiffening of central arteries may be the cause for ISH. Mechanism that is causing arterial stiffening are many . There are evidences which show increased homocyteine causes impaired nitric oxide mediated relaxation of vessel wall and increased smooth muscle cell proliferation . Other interesting mechanism may be stimulation of elastinolytic process in the arterial wall thereby causing increased stiffness of arterial wall .

Homocysteine and renal disease

Kidneys play a vital role in homocysteine metabolism. Patients with

Failing kidney , terminal kidney disease and high concentration of urea in blood have increased concentration of homocysteine and are having more propensity towards development atherosclerosis.

Homocysteine and diseases of vessels of heart [23]

Seventeen studies were evaluated were conducted regarding homocysteine and cardiovascular disease , out of which in 14 studies, homocysteine was found to be a significant risk factor premature atherosclerosis and thrombus formation . It was

calculated that 10% of all Coronary heart disease(CAD)risk in population was due to elevated homocysteine.

It was observed that a 30-40% reduction in risk of CAD by long term lowering of homocysteine level by 3-4 umol/lit

Study done by Boushey et al.[24] based on meta analysis of 27 studies shown that an increased risk of Coronary heart disease ,peripheral vascular disease and venous thrombosis and embolism with an increase in homocysteine concentration 15 μmol/L or greater

Arnesen et al,[25] showed a significantly positive correlation between coronary artery disease and homocysteine. In their case control study of 21,656 subjects (11-61 years old), 123 developed ischemic heart disease within a mean follow up period of four years. Homocysteine levels in cases were found to be significantly higher than that in controls. Thus, homocysteine can be attributed as independent risk factor.

Nygard et[26] al studied 587 angiographically documented cases. They reported a

association between raised homocysteine and mortality in manner that depends on concentration of homocsteine . According to them as homocysteine concentration increases severity of diseases increases .

Chacko studied patients with CAD in Asian Indians and 53 control subjects. They concluded that homocysteine is not a major risk factor for coronary heart disease in Asian Indians.[27]

Chambers et al[28]. studied UK Indian Asians with CAD and compared them with Europeans having CAD. They studied 764 male patients (257 Indian Asian and 507 European). Their results revealed that plasma homocysteine concentrations were higher in Indian Asians compared with Europeans.

Ford et al,[29] analyzed the prospective trails and concluded that there was 20% increase in cardiovascular risk for every 5 μmol/L increase in homocysteine levels Enas A Enas et [30] al found that women with homocysteine levels more than 15.6 μmol/L had twice the risk of Myocardial infarction than the women with homocysteine levels <10μmol/L.

Stampher et al,[31] In Physicians health study studied 14,916 male physicians without known atherosclerosis. Men are susceptible to myocardial infarction three times more compared to normal people when their plasma concentration increases 12% more than normal level .

Homocysteine and coronary artery disease in young adults

Myocardial infarction is claiming a large number of lives in young patients. Various studies have shown that homocysteine may be significant marker of risk in young patients lacking conventional risk factors .

In a case control study by ogawa et al, [32] 127 men with early myocardial infarction (<45 years) and 150 age matched controls and concluded that hyperhomocysteinemia is an independent risk factor for myocardial infarction.

Khare A et al.[33] studied homocysteine levels in 120 patients with myocardial infarction less than 40 years age. They concluded that elevated homocysteine levels were independently associated with CAD.

VK Katyal et al.[34], studied 100 young patients(less than 40 years) admitted with acute MI and found hyperhomocysteinemia to be a significant contributor towards premature CAD.

Study conducted by Puri A et al[35]. involving 51 patients less than 45 years of age with CAD plasma homocysteine emerged as a significant independent risk factor for young CAD patients.51

Stephen M Schwartz et al[36] studied 79 women of age < 45 years diagnosed with MI and concluded that elevated plasma homocysteine and low plasma folate are risk factors for MI among young women.

Homocysteine and pregnancy [37] :

Studies are limited .Sunita Ghike et al, in their study show patient with severe pre eclampsia are found to have increased serum homocysteine value and severity correlate with increased homocysteine level . Foetal complications are more in patients with increased homocysteine in mother in the study group compared to normal control.

Other studies points towards elevated homocysteine level in pregnant women was associated increased miscarriage and fetal congenital malformations . They also emphasize on supplementation of vitamin B12 and folic acid in preventing fetal malformation . Exact correlation between hyperhomocysteinemai in pregnancy and its effects needs further study .

TREATMENT

For reducing plasma level of homocysteine the very successful, efficient and easy way is administering the patients with vitamin supplements like folic acid , vit B12 , and vitamin B6 . A strong negative correlation between folic acid level in the body and the plasma homocysteine levels has been reported.

The guidelines of the American heart association advocate the principle of screen and treat, i.e., screening for hyperhomocysteinemia is recommended only in high risk population (personal or family history of premature atherosclerosis,myocardial infarction, hypertension, diabetes).

It is recommended [38]to keep the homocysteine levels to <10μmol/L in the high risk group. Those with hyperhomocysteinemia should be treated with dietary modification followed by vitamin supplementation or fortification of food with vitamins (400 μgm of folic acid, 2mg of vitamin B6 and 6μgm of vitamin B12).

In a trial aimed at stroke prevention by vitamin supplementation [39], patients with stroke and higher homocysteine levels were randomly allotted into two category , one category received heavy dose of vitamin supplementation and other category received low dose of vitamin supplementation . There was reduction in the homocysteine level 2umol/litre more in the heavy dose receivers compared to low dose receivers . But there was no significant

differences in the reduction of cerebrovascular events , coronary diseases and mortality after following the patients for two years .

Results have shown that folic acid supplementation reduced plasma homocysteine by 41%, whereas Vit B12 supplement lowered homocysteine level by 14.8% and both were significant. The daily vitamin B6 did not significantly reduce plasma homocysteine level. The combination of three vitamins reduced plasma homocysteine by 49.8%. [39,]

Chronic kidney disease and homocysteine :

Normal handling of homocysteine by kidneys [40,41] :

It is now known that kidney play a vital role in metabolism of aminoacid. Unbound aminoacids are filtered at glomerulus freely . Those filtered level indicates the serum aminoacid level and glomerular filtration rate .Around 450mmol of aminoacids are filtered daily. More than 90% are reabsorbed in proximal convoluted tubule and only around 5mmol per day excreted in urine .

Tubular cell basolateral surface plays a minor role in uptake of aminoacid which mainly occurs in distal tubule cell .Homocysteine is probably taken up by this mechanism .

Homocysteine metabolism in kidney [42]:

As with other aminoacids kidney plays a role in metabolism of homocysteine. Homocysteine has a molecular weight of 135 D which comes into the filtration limit of renals .

Assuming plasma free homocysteine level 3 umol and normal glomerular filtration rate of 125 ml/min daily amount of homocysteine filtered will be around 0.5 mmol .[43]

As with other amino acid homocysteine is freely filtered at glomerulus , reabsorbed at the tubules and minimally excreted .

Specific mechanisms for reabsorption of homocysteine at tubular level have been recognized . Studies in mouse models have been found to have different channels for absorption including low km/high affinity ,high km/low affinity homocysteine uptake system . Low km/high affinity system shares process with cysteine and othe dibasic aminoacids like arginine,ornithine , lysine [43,44.]

Humans and rats[45] show increased excretion of urinary homocysteine after bolus injection of aminoacid arginine and lysine or alpha aminoisobutyric acid an inhibitor of lysine ,ornithine and arginine tubular reabsorption . Enzymes required for transulfuration (Cystathionine B synthase and

cystathionase) and remethylation (Methionine synthase) in human predominantly found in kidney and liver .

Some hypothesis says that kidney will alter its function so as to adjust for changes in GFR by increasing or decreasing these two above said biochemical pathways . According to this theory an elevation in glomerular filtration rate will cause an elevated homocysteine filtration and tubular absorption [46.] According to it increase in GFR will cause an upregulation homocysteine metabolizing enzymes. On the opposite hand if GFR decreases intrarenal homocysteine metabolism will fall . These adoptive mechanisms make sure that alteration in homocysteine ultrfiltration do not affect renal homocysteine transportation to plasma[47] . Healthy kidney has the capacity to filter, reabsorb,and metabolise homocysteine In addition to filtered load homocysteine uptake also occur on the basolateral tubular cell surface .

Arteriovenous studies[48] have been performed on normal human rat kidney to know the capacity of an organ to extract the homocysteine . Homocysteine concentration on the aterial side and venous side are measured after considering of kidney flow of plasma and loss of the homocysteine in the urine . These studies have shown that kidneys are capacitant enough of extracting homocysteine in very high amounts . Limitation in this study was this did not take into account of the homocysteine produced by kidney.

Food intake and its effect on homocysteine filtration[49]:

Studies conducted on this have shown that dietary intake affects homocysteine binding ,and thereby homocysteine fitration at glomerulus .One published data shows that there is significant increase in homocysteine level after a protein rich meal with a peak level at 8 hour . Free homocysteine level increase was more significant than bound homocysteine .These experiments help to explain increase or decrease in homocysteine level based on dietary habits[50] . Food containing high amount of protein like methionine will lead to increase in plasma homocysteine level and cysteine level .Competition for protein binding and disulfide exchange reactions cause a disproportionate increase in free homocysteine level .This causes excess free homocysteine to be delivered to kidney for filtration . Increase in free homocysteine also enhance uptake by tissues other than kidney . Both mechanisms including excessive filtration of homocysteine at the kidney level and upregulation of homocysteine degradation pathway in the kidney will lead to increased removal of homocysteine from the plasma .

Recent data using N-acetyl cysteine (NAC) in acetaminophen overdose shows[51] some interesting finding regarding protein binding and metabolism of homocysteine.

N -acetyl cysteine interacts with sulfhydril containing substances, also interacts with cysteine and homocysteine displacing from other protein binding sites and forming mixed disulfides . Human subjects treated with NAC either oral form or intravenous form shows significant often dramatic and dose dependent fall in homocysteine and cysteine level . NAC administration also increases the free versus bound homocysteine . These findings show that alteration in binding of homocysteine to protein of homocysteine protein binding by drugs or or diet can temporarily cause elevation of free homocysteine level thereby increasing glomerular filtration and renal metabolism

Diseased kidney and homocysteine handling[52] :

Correlation between GFR and homocysteine levels :

Studies conducted till now based on presumption that normal kidneys plays important and efficient role in homocysteine handling , homocysteine level increases as renal function deteriorates, reaching maximum level in the end stage renal disease(ESRD) , with majority of CKD patient in ESRD on dialysis having significant elevation of homocysteine values .

Glomerular filtration values calculated from serum creatinine or calculated creatinine clearance show inverse relationship with homocysteine values .It

is not sure however whether creatinine inversely predicts homocysteine levels because it is a marker of GFR or simply because it is linked to homocysteine production via a common biochemical pathway. Many studies have been conducted using highly accurate GFR measurement in healthy and diabetic patient . These studies showed strong inverse relationship between homocysteine levels and renal function . This has been proved even with creatinine and more sensitive markers like cystatin C . In conclusion homocysteine and renal function having an inverse relationship . It has been noted [53] using multiple markers of GFR markers over a broad spectrum of renal function from severe kidney dysfunction to a stage very high above the the uremic range.This indicates that increased homocysteine level in kidney diseases are highly linked with function of renal system .

Binding to plasma protein in kidney disease[54] :

Free homocysteine , bound homocysteine and total homocysteine levels are elevated in kidney disease even though proportion of free homocysteine remains constant or decreases .In CKD patients in uremic patients who are frequently hypoalbumenic have homocysteine bound to protein more compared to free homocysteine. It is hypothesized that retained uremic plasma proteins play a major role in binding homocysteine thereby increasing proportion of bound homocysteine concentration .Further studies are going on to find out proportion of free homocysteine, amount of

toxins in uremia and its effect on homocysteine binding and potential toxicities of reduced homocysteine, free homocysteine, bound homocysteine in uremic environment

Metabolism of aminoacids in Chronic kidney disease[55] :

Aminoacid handling ,metabolism and elimination are significantly altered in uremic environment .It may be because of uremia induced malnutrition or because of retained toxins, hormonal level derangement ,may be because of increased urinary amino acid excretion or may be because of changing capacity of kidney to clear ,synthesize and degrading certain aminoacids . Due to reduction in plasma level of certain essential aminoacid like isoleucine , leucine valine , lysine and increases in levels of citrulline , cystine ,histidine ,glycine ,hydroxyproline and total non essential aminoacid there will be alteration in synthesis and amount products from these precussors .In normal persons excretion of total aminoacid is minimal it increases with progressive decline in renal function. This is due to higher plasma concentration of some specific aminoacid leading to increase in filtered load and and subsequent filtrate flow rate in few remaining functioning nephrons .This leads to decreased transit time and contact time between amino acid and tubule cells leading decreased metabolism of these aminoacids and and their increased excretion in urine .

Renal and extra renal metabolism of homocystine in Chronic kidney disease:

Hyperhomocysteinemia in chronic kidney disease is due to defective clearance of homocysteine from decreased functioning of kidney . It is not due to increased delivery of plasma homocysteine .Kinetics studies[56] were performed using oral and intravenous load of homocysteiney disease patient in chronic kidne showed that clearance of homocysteine reduced in these patients compared to normal healthy subjects and subjects with Vit B 12 and folate deficiencies .This study shows that increase in homocysteine levels in these CKD patients are due to decreased clearance from malfunctioning kidney and decreased extrarenal metabolism caused by retained uremic solutes . At present there is no clear cut additional data to indicate that what level of defective metabolism is responsible for increase in plasma homocysteine level in CKD patients . Existence of homocysteine metabolizing enzymes and other aminoacid metabolizing enzymes have been identified in renal tubular epithelial cells . Homocysteine extraction studies in animals have proven significant uptake of homocysteine by tubular epithelial cells . With this studies it appears that loss of functional renal mass leads to decreased renal excretion of homocysteine and their level increase in plasma . There appears inverse relationship between renal GFR and plasma homocysteine level as proven by many studies .

An alternate theory[57] in chronic kidney disease and plasma homocysteine elevation is accumulation of uremic toxins and decreased extrarenal metabolism of homocysteine . The exact substances till now not identified , Liver appears the major target organ as it is involved in metabolism of majority of aminoacids in body . Liver additionally has got high level of homocysteine metabolizing enzymes so it plays major role in extrarenal homocysteine metabolism . In vitro studies have shown defective transport of folic acid in uremic mileu. Further studies in CKD paients showed no defect in absorption ,transport and conjugation of homocysteine . In another study in rat it showed abnormal metabolism of sulphur containing aminoacid in uremic mileu

Van Guldener et al [58,] literature on homocysteine metabolism in chronic renal failure states that there are two options available to explain increased homocysteine levels in kidney failure patients .One is defective clearance or metabolism by kidney itself .Another mechanism is systemic impairement of whole body homocysteine metabolism . .In that article they state that urinary homocysteine excretion in human is minimal , defective excretion itself cannot explain hyperhomocysteinemia in CKD patients . According to them loss of intratubular metabolism of homocysteine is main factor contributing to hyperhomocysteinemia in CKD patients.

Systemic factors may contribute[59] to hyperhomocysteinemia in CKD patients . Including among those are deficiencies of vitamins like folate , vit B12 , vit B6 , genetic factors and altered total body homocysteine turnover . Role of vitamins can be studied by measuring plasma vitamin levels and measuring the homocysteine level after vitamin supplementation . But in CKD patients it was found that levels of vitamins are within normal range , but apparently insufficient to prevent hyperhomocysteinemia .But relation between vitamin level and homocysteine are maintained but at higher level than with the patients of normal renal function .

Of the three vitamins, folic acid[60] is the main vitamin playing vital regulatory role in homocysteine metabolism and strongest determinant of plasma homocysteine level in CKD patients . Study conducted have shown that only folic acid containing therapy is able to lower the plasma homocysteine level in chronic renal failure patients . Cobalamin is found to be effective only when Vit B 12 level is low.

Vit B6 found no significant effect on fasting plasma homocysteine level . Some studies point toward the presence of serine deficiency as a contributing factors in development of hyperhomocysteinemia as it is contributing in both transulfuration and in the folate cycle and serine level found low in chronic renal failure patients . Betain dependent remethylation defect also possible for hyperhomocysteinemia

But in CKD patient betain level found to be normal and treatment with betain alone or added to folic acid found not to reduce homocysteine level .

Till now so specific genetic [61] defect responsible for hyperhomocysteinemia in chronic renal failure patient has been identified . Those patient in which there is defect in 5, 10 methyl tetrahydrofolate reductase with CKD have found higher level of homocysteine than isolated defect in enzyme without renal dysfunction .

Oral methionine and homocysteine loading and stable isotope test have shown the possibility of third mechanism of hyperhomocysteinemia.

To elucidate[62] exact cause of hyperhomocysteinemia in patients with CKD patients several studies involving methionine , homocysteine loading test , stable isotope test were conducted . According to this , patients with CKD after oral methionine loading test exhibit exaggerated plasma homocysteine level suggesting a transulfuration defect . In hemodialysis patients post methionine loading plasma homocysteine was mainly due to deficiency of serum folate not due to Vit B 12or Vit B 6 . Folate therapy lowered both fasting and post methionine homocysteine level indicating impairement of folate dependent remethylation defect in chronic renal failure and interdependency between methylation and transulfurtion .

After a homocysteine loading orally ,renal failure patients exhibited three to four fold increase in the plasma homocysteine half life compared with normal patients .

Stable isotope study [63]conducted to quantitatively assess whole body transmethylation , remethylation ,transulfuration have shown strongly towards defective total body remethylation as the reason for increased homocysteine level in kidney failure patients . Pathogenesis of this process of impairement is not known . But presumed to be due to direct inhibitory effects of uremic toxin or to an altered folate metabolism in uremia .

Homocysteine lowering trials in Chronic kidney disease patients

Continuous efforts are being done to decrease homocysteine level in spatients with renal failure many studies are conducted .Results of those studies have shown that in contrast to normal people in renal failure patients hyperhomocysteinemia is often resistant to all available treatment like folic acid , Vit B 12 , Vit B6, betain , serine . This was seen uniformly in all patients with CKD . Refractoriness increases as renal function deteriorates . Hyperhomocysteinemia in CKD patients frequently becomes normal with supraphysiological doses of folic acid such as 2 to 15 mg per day. These were basesd on study in dialysis and renal transplant patient who are often found to have low folate and other vitamin levels .

This causes some kind of confounding in treatment of CKD patients with increased homocysteine . Patient with end stage renal disease will respond to supraphysiological doses of vitamins which show that even in ESRD some functions of kidney are maintained and that is the reason they respond to high doses but in an attenuated manner .Alternatively uremic solutes effectively inhibit extrarenal metabolism .

Studies are concentrated towards presence of genetic pomorphism in patients with CKD which are contributing to increased homocysteine level particularly methylenetetrahydrofolate thermolabile [64] mutation . These studies have shown presence of hyperhomocyasteinemia only in presence of low folate level .

Other studies by Ziad A Massy[65] in his article therapy of homocysteinemia in chronic renal failure patients states that folic acid therapy in patients with CKD has shown to reduce but not normalizing the increased homocysteine level .Resistance to folate therapy shows that correlation between folic acid and homocysteine only partial .Routine 1mg folic acid treatment often ineffective in patients with CKD . Oral supplementation of folic acid with very high doses around 15mg daily causing 20 to 50 fold increase in plasma folate concentration only partially correcting homocysteine level. Mechanism of resistance is not due to defective absorption or due to defective conversion into active form 5 – methyl tetrahydrofolate .

Alternatively reduced forms of folic acid which are metabolically more active than naïve folic acid was not associated with decreased homocysteine level to larger extent . Folic acid supplementation did not increase homocysteine clearance in CKD patients . Interpretation of all these study findings are eventhough folic acid increases homocysteine remethylation in tissues and decreases export of homocysteine from tissues into plasma compartment it is not possible to overcome the primary defect of reduction in homocysteine clearance from plasma .

Other mechanism which causes resistance to treatment in patients with CKD are resistance to Vit B 12 causing abnormalities of remethylation pathway .Elian and Hoffer found that by administering physiological doses of Vit B12(1mg per week) in patients with CKD on hemodialysis who were already receiving folate 5 to 6mg per day and vitamin B6 5mg per day to increase concentration of Vit B 12 TO 50 to 60 fold showed to decrease the plasma homocysteine level by 32 % less than the lowest lowel attained by treatment with high doses of folate and pyridoxine .

Homocysteine in CKD Patients and dialysis [66]:

Accumulation of uremic toxins and decrease in homocysteine clearance and metabolism due to decreased functioning kidney mass are main cause of hyperhomocysteinemia in dialysis patients .Some studies have pointed that patients with CKD without dialysis are found to respond

to therapy with folic acid in contrast to those who are on dialysis. It depends on type of dialysis and dialyser used . Most efficient dialyser remove uremic toxins and homocysteine and improves outcome .

Low flux dialyzers[67] are unable to remove sufficient toxins and homocysteine . High flux dialyzers are also found not to decrease homocysteine level to much extent . In contrast superflux mode significantly decreased homocysteine level compared with high flux mode .This may be due to removal of albumin with which homocysteine in bound . Recent study show that patients who are undergoing daily nocturnal hemodialysis are found to have low homocysteine values compared to those with routine hemodialysis . This effect was supposed to be due to better removal of small and middle molecules in nocturnal hemodialysis patients .

Studies are going on to displace bound homocysteine by using N acetyl cysteine before hemodialysis [68] and subsequent removal using hemodialysis .Acute intravenous infusion of NAC [69] 5gm in 5% dextrose over 4 hours during a hemodialysis session can displace bound homocysteine and was able to normalize total homocysteine concentration at the end of dialysis session with residual effect up to two days . Even though this approach was found useful, long term safety and efficacy of NAC need to be evaluated before drawing definite conclusion . On the other hand long term oral NAC at 1.2gm two times daily was not found to reduce homocysteine level in

dialysis patients . This area of removal of homocysteine [70]using dialysis with additional use of NAC is an areas of research aimed at decreasing homocysteine level and treatment strategy needs further studies

MATERIALS AND METHODS

PLACE OF STUDY :

Thanjavur medical college and hospital ,Thanjvur

TYPE OF STUDY:

Single centred Prospective and observational

PERIOD OF STUDY :

Study conducted during a period of 8 months from January 2014 to August 2014

ETHICAL COMMITTEE APPROVAL :

Present study conducted was approved by institutional ethical committee :

COLLABORATING DEPARTMENT :

Department of General medicine and department of nephrology

CONSENT:

Informed consent was obtained from the patient to participate in this study

SAMPLE SIZE:

50 patients of chronic kidney disease who admitted to Thanjavur medical college and hospital were chosen for study meeting inclusion and exclusion criteria

SELECTION OF PATIENTS :

50 patients satisfying following inclusion criteria and not having exclusion criteria were taken up for the study

INCLUSION CRITERIA :

Patients with

1.Elevated blood urea and serum creatinine

2. Ultrasound abdomen showing features of chronic kidney disease

EXCLUSION CRITERIA :

Patients with

1. Acute kidney injury
2. Patients who are current smokers
3. Patients who are current alcoholics
4. Patients who are known case of chronic liver disease
5. Patients who are having diabetes mellitus

STUDY METHOD :

50 patients with chronic kidney disease who are admitted in Thanjavur medical college for symptoms related to chronic kidney disease or other reasons are selected for this study . Personal characteristics like age, sex , weight height, address were noted . Patients were enquired for presence of family history of chronic kidney disease or whether patient is undergoing dialysis for the chronic kidney disease, presence or absence of diabetes mellitus , systemic hypertension , chronic liver disease , smoking habits or alcohol consumption . General and systemic examinations done

INVESTIGATIONS :

Random blood sugar

Blood urea ,serum creatinine

Serum sodium , potassium

Complete hemogram

Liver function test

Eletrocardiogragh

Ultrasonography abdomen

Plasma homocysteine

Glomerular filtration rate (calculated)

Fasting Plasma homocysteine were measured by flouroscein polarization immunoassay

Obtained data and results are analysed by calculating percentage mean values standard deviation and standard error using T Test , chi –square test. All statistical calculations are done using the software SPSS for windows 14 evaluation version and conclusion drawn

OBSERVATION AND RESULTS

Age wise distribution of patients :

Of the 50 patients of chronic kidney disease selected majority of patients were in age group between 41 to 60 years . From this table it is pbvious that as age increases the incidences and prevalences of people suffering from chronic kidney disease increases. But because of limited sample size exact prevalences among different age group cannot be arrived . As age increases the functioning nephron number decreases leading to chronic kidney disease(table.1)

Age distribution of patients :

Table 1

Age	Number of patients	Percentage
<20	1	2%
21-30	1	2%
31-40	4	8%
41-50	13	26%
51-60	21	42%
>60	10	20%

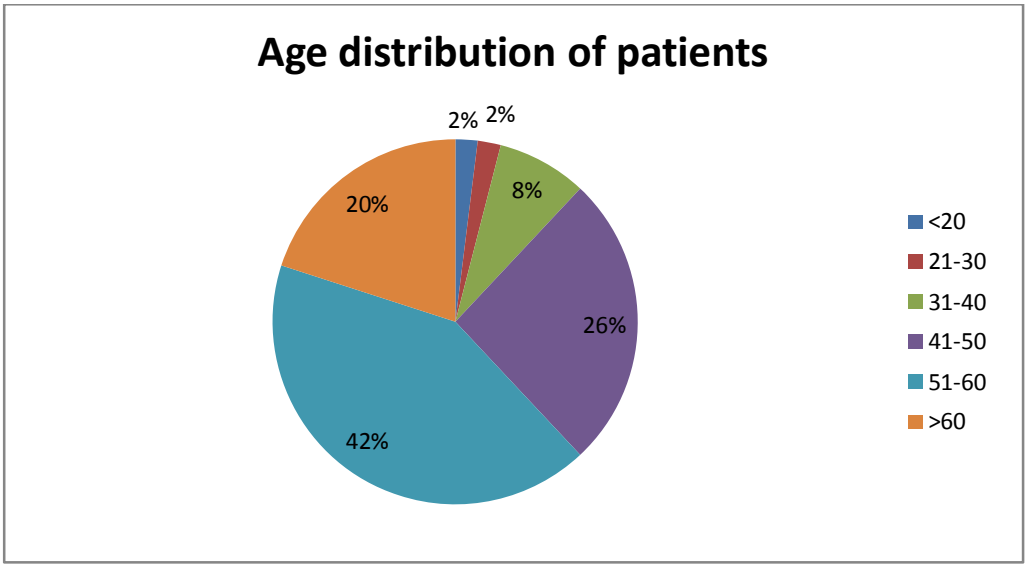


Figure 1

Sex distribution of patients :

Of the patients selected for the study 37 patients were males and 13 patients were females (table 2,figure 2)

Table 2

Males	37	74%
Females	13	26%

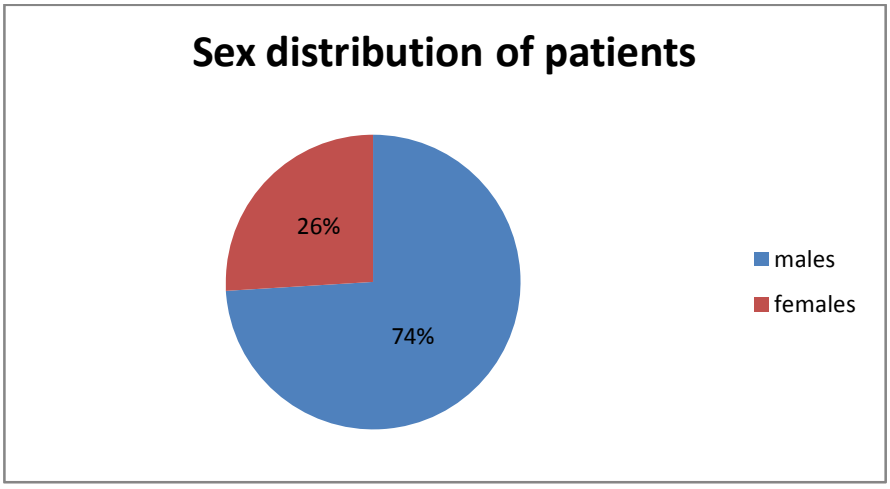


Figure 2

Staging of CKD according to creatinine clearance and glomerular filtration rate as calculated by cockcroft gault formula :

Of the patients selected majority of patients in CKD stage 4 and 5 constituting around 94 percentage (table 3 , figure 3)

Table 3

Stage of CKD	Number of patients	Percentage
0	0	O
1	O	O
2	0	0
3	3	6
4	11	22
5	36	72

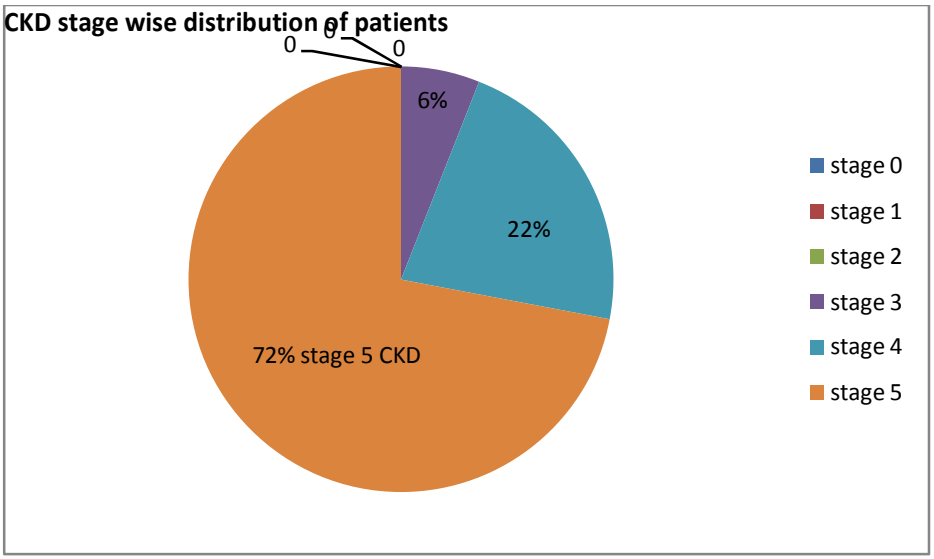


Figure 3

HYPERHOMOCYSTEINEMIA:

Of the 50 patients in the study 39 patients found to be having elevated plasma Homocysteine values constituting around 78% (table 4,figure 4)

Table 4

Sex	Total number	Normal HCY	HyperHCY	Percentage hyper HCY
Males	37	9	28	75%
Females	13	2	11	84%

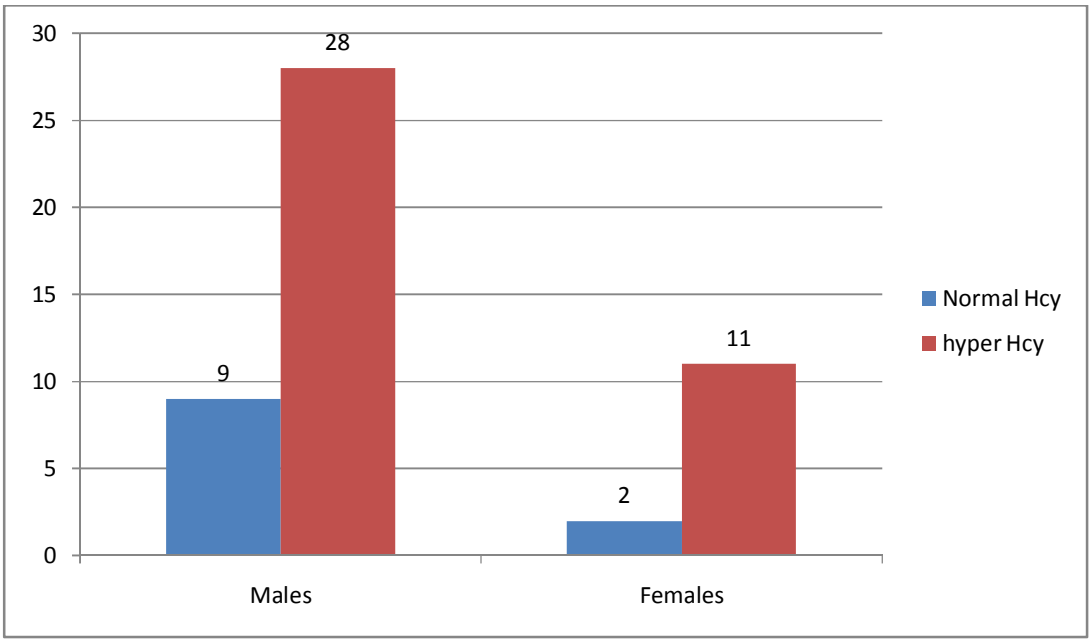


Figure 4

Stage of CKD and elevated homocysteine level:

If we compare plasma homocysteine level with corresponding glomerular filtration rate and stage of CKD it shows that as patient deteriorates to next lower level of chronic kidney disease incidence of hyperhomocystenemia increases . Here in this study we observed that in stage 4 and stage 5 of chronic kidney disease incidence of hyperhomocysteinemia were 72% and 96.67 percentage respectively.

Table 5

Stage of CKD	Number of patients	Normal HCY	Hyperhomo Cystenemia	Percentage of hyperhomocystenemia
0	o	0	0	0
1	0	0	0	0
2	0	0	0	0
3	3	1	2	66.6%
4	11	3	8	72.72%
5	36	7	29	80.55%

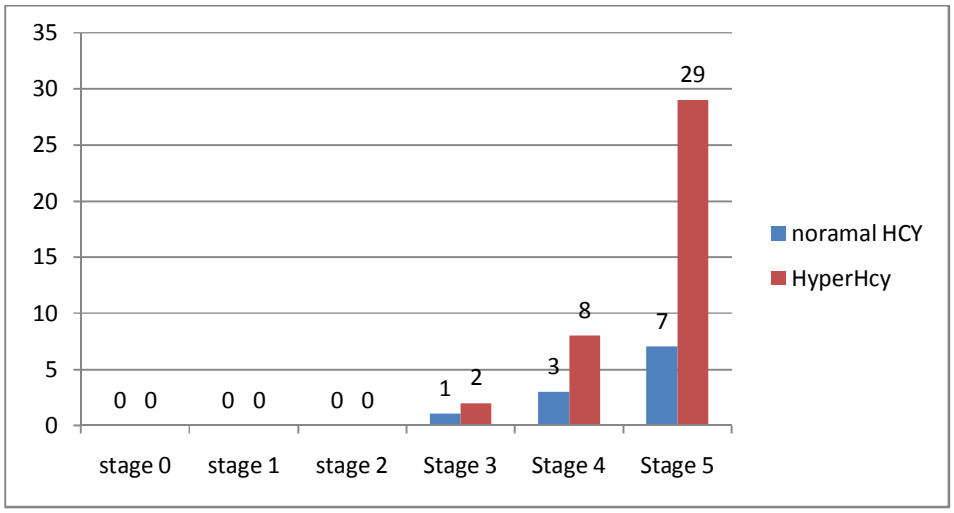


Figure 5

ECG finding in CKD patients with normal and increased homocysteine level :

We observed that majority of patients were having abnormal electrocardiograph indicating majority of patients with Chronic kidney disease were having some kind of cardiac diseases emphasizing the fact that cardiovascular diseases are main causes of morbidity and mortality in CKD patients . Majority were having left ventricular hypertrophy , may be because of increased prevalence of hypertension in patients with Chronic kidney disease . Of the 50 patients selected for the study 47 patients were having abnormal electrocardiograph constituting 94% . In those with hyperhomocysteinemia only three were found to have normal ECG . All others were having abnormal ECG (table 6 ,figure 6)

Table 6

ECG	HOMOCYSTEINE	
	NORMAL	INCREASED
	11	39
NORMAL	0	3
ABNORMAL	11	36
PERCENTAGE OF ABNORMAL ECG	100%	92.6%

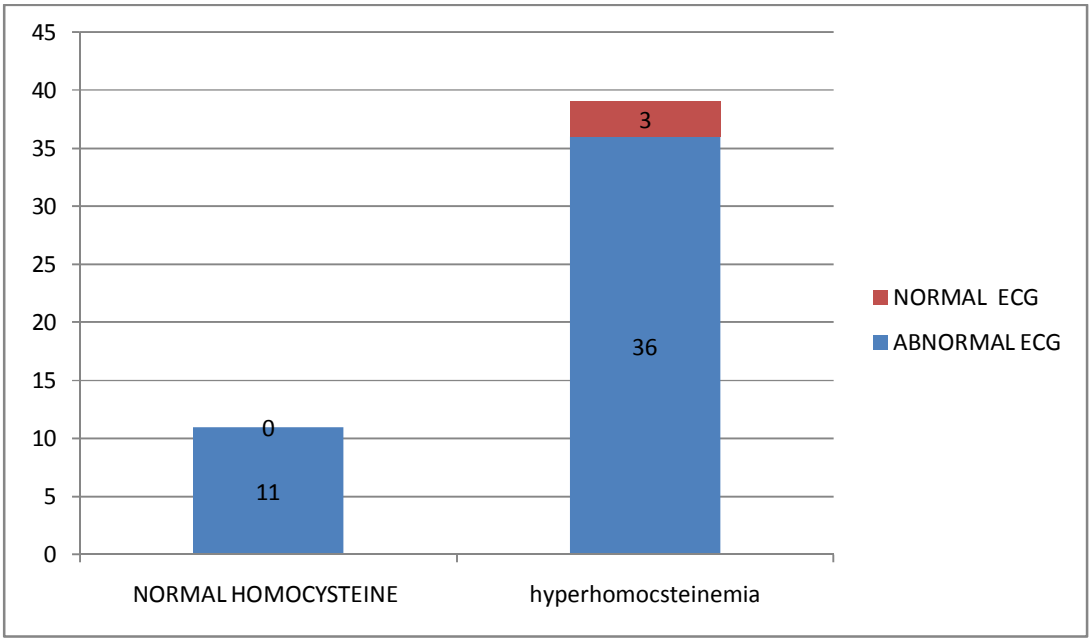


Figure 6

Hyperhomocysteinemia is classified into

Mild , level between 15 -30 umol/lite

Moderate 31- 100 umol/litre

Severe > 100 umol/litre .

In our study in those having hyperhomocysteinemia majority are falling in the group of mild hyperhomocysteinemia . Of the 39 patients with hyperhomocysteinemia 37 patients were in the group of mild hyperhomocysteinemia . Only two were found to have moderate hyperhomocysteinemia .(table 7, figure 7)

Table 7

Hyperhomocysteinemia	Numbers	Percentage
Mild	37	94.87%
Moderate	2	5.1%
Severe	0	0%

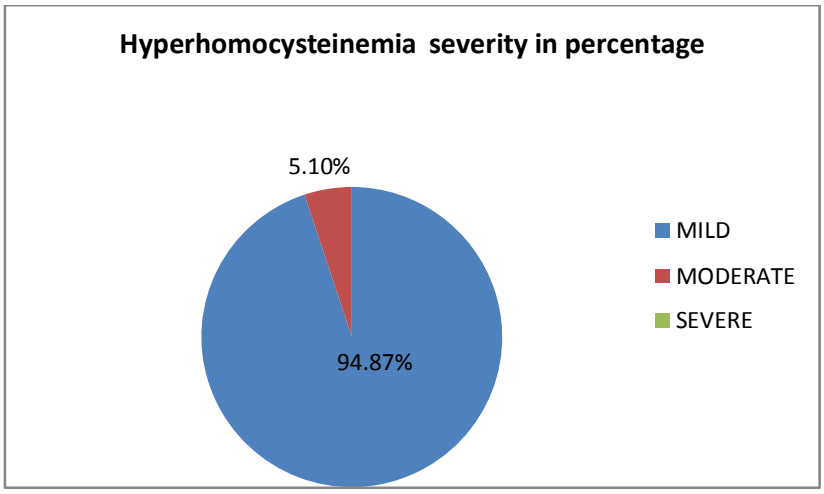


Figure 7

In our study there were 7 patients with CKD were on intermittent hemodialysis or peritoneal dialysis . But all were found to have mild hyperhomocystinemia

We excluded the patients with diabetes mellitus because diabetes itself can increase the homocysteine by other mechanisms .So it may act as a confounding factor .

In our study we found that of the 50 patients selected for study 35 had hypertension . Of the 35 patients 27 had elevated homocysteine level . Those without hypertension were 15 patients . Among them 12 had elevated homocysyeine level (table 8, figure 8)

Table 8

Hypertension	Homocysteine	
	Normal(11)	Elevated (39)
Present	8(72.7%)	27(69.2%)
Absent	3(27.2%)	12(30.7%)

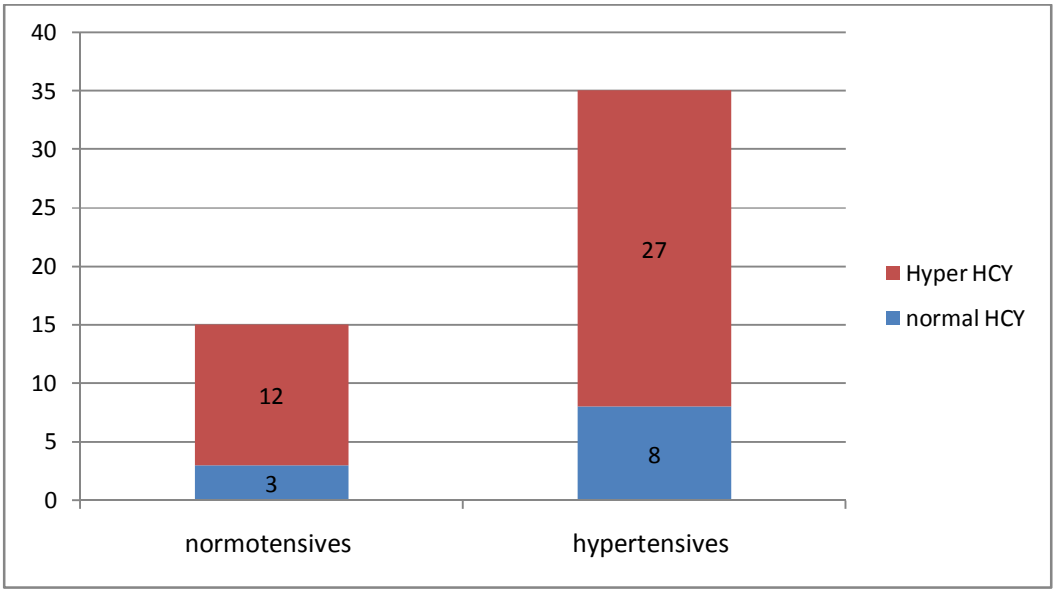

40
35
30
25
20
15
10
5
0
12
3
27
8
normotensives
hypertensives
Hyper HCY
normal HCY

Figure 8

Statistical analysis of this study:

Frequency Table

Age *(years)*

Particulars	No.of respondents (n=50)	Percentage (100%)
Below 40yrs	6	12.0
41 to 50yrs	13	26.0
51 to 60yrs	21	42.0
61 to 70yrs	8	16.0
71yrs & above	2	4.0

Sex

Particulars	No.of respondents (n=50)	Percentage (100%)
Male	37	74.0
Female	13	26.0

Weight(Kg)

Particulars	No.of respondents (n=50)	Percentage (100%)
40 to 49kgs	10	20.0
50 to 59kgs	16	32.0
60 to 69kgs	14	28.0
70 to 79kgs	9	18.0
80kgs & above	1	2.0

Diabetes mellitus

Particulars	No.of respondents (n=50)	Percentage (100%)
Absent	50	100.0

SHT

Particulars	No.of respondents (n=50)	Percentage (100%)
Absent	15	30.0
Present	35	70.0

ECG

Particulars	No.of respondents (n=50)	Percentage (100%)
No	3	6.0
Ab	47	94.0

on dialysis

Particulars	No.of respondents (n=50)	Percentage (100%)
No	43	86.0
Yes	7	14.0

GFR(ml/min)

Particulars	No.of respondents (n=50)	Percentage (100%)
Less than 15	35	70.0
15 to 29	12	24.0
30 to 59	3	6.0

Homocysteine (umol/lt)

Particulars	No.of respondents (n=50)	Percentage (100%)
Negative	11	22.0
Positive	39	78.0

Descriptive Statistics

Item	Min.	Max.	Mean	S.D
Age(years)	18	75	53.10	11.151
Weight(Kg)	40	100	57.82	11.549
Urea(mg%)	60	204	113.66	32.128
Creatinine(mg%)	2.00	22.20	7.4380	4.47984
GFR(ml/min)	2.43	33.46	12.8300	6.95518
Homocysteine(umol/lt)	9.34	58.00	19.6740	7.83157

Chi-square test

Homocysteine(umol/lt)

Age(years)	Negative		Positive		Total		Statistical inference
	(n=11)	*(100%)*	*(n=39)*	*(100%)*	*(n=50)*	*(100%)*	
Below 40yrs	1	9.1%	5	12.8%	6	12.0%	X^2=1.427 Df=4 .840>0.05 Not Significant
41 to 50yrs	2	18.2%	11	28.2%	13	26.0%	
51 to 60yrs	5	45.5%	16	41.0%	21	42.0%	
61 to 70yrs	2	18.2%	6	15.4%	8	16.0%	
71yrs & above	1	9.1%	1	2.6%	2	4.0%	

Chi-square test

Homocysteine(umol/lt)

Sex	Negative		Positive		Total		Statistical inference
	(n=11)	*(100%)*	*(n=39)*	*(100%)*	*(n=50)*	*(100%)*	
Male	9	81.8%	28	71.8%	37	74.0%	X^2=.448 Df=1 .503>0.05 Not Significant
Female	2	18.2%	11	28.2%	13	26.0%	

Chi-square test

Homocysteine(umol/lt)

Weight(Kg)	**Negative**		**Positive**		**Total**		**Statistical inference**
	(n=11)	***(100%)***	***(n=39)***	***(100%)***	***(n=50)***	***(100%)***	
40 to 49kgs	1	9.1%	9	23.1%	10	20.0%	X^2=7.657 Df=4 .105>0.05 Not Significant
50 to 59kgs	5	45.5%	11	28.2%	16	32.0%	
60 to 69kgs	1	9.1%	13	33.3%	14	28.0%	
70 to 79kgs	3	27.3%	6	15.4%	9	18.0%	
80kgs & above	1	9.1%	0	.0%	1	2.0%	

Chi-square test

Homocysteine(umol/lt)

DM	**Negative**		**Positive**		**Total**		**Statistical inference**
	(n=11)	***(100%)***	***(n=39)***	***(100%)***	***(n=50)***	***(100%)***	
Absent	11	100.0%	39	100.0%	50	100.0%	-

Chi-square test

Homocysteine(umol/lt)

SHT	**Negative**		**Positive**		**Total**		**Statistical inference**
	(n=11)	***(100%)***	***(n=39)***	***(100%)***	***(n=50)***	***(100%)***	
Absent	2	18.2%	13	33.3%	15	30.0%	X^2=.938 Df=1 .333>0.05 Not Significant
Present	9	81.8%	26	66.7%	35	70.0%	

Chi-square test

Homocysteine(umol/lt)

ECG	Negative		Positive		Total		Statistical inference
	(n=11)	*(100%)*	*(n=39)*	*(100%)*	*(n=50)*	*(100%)*	
No	0	.0%	3	7.7%	3	6.0%	X^2=.900 Df=1 .343>0.05 Not Significant
Ab	11	100.0%	36	92.3%	47	94.0%	

Chi-square test

Homocysteine(umol/lt)

on dialysis	Negative		Positive		Total		Statistical inference
	(n=11)	*(100%)*	*(n=39)*	*(100%)*	*(n=50)*	*(100%)*	
No	11	100.0%	32	82.1%	43	86.0%	X^2=2.296 Df=1 .130>0.05 Not Significant
Yes	0	.0%	7	17.9%	7	14.0%	

Chi-square test

Homocysteine(umol/lt)

GFR(ml/min)	Negative		Positive		Total		Statistical inference
	(n=11)	*(100%)*	*(n=39)*	*(100%)*	*(n=50)*	*(100%)*	
Less than 15	7	63.6%	28	71.8%	35	70.0%	X^2=.369 Df=2 .831>0.05 Not Significant
15 to 29	3	27.3%	9	23.1%	12	24.0%	
30 to 59	1	9.1%	2	5.1%	3	6.0%	

Chi-square test

GFR(ml/min)

Age(years)	Less than 15		15 to 29		30 to 59		Total		Statistical inference
	(n=35)	*(100 %)*	*(n=12)*	*(100 %)*	*(n=3)*	*(100 %)*	*(n=50)*	*(100 %)*	
Below 40yrs	5	14.3 %	1	8.3%	0	.0%	6	12.0 %	X^2=3.417 Df=8 .906>0.05 Not Significant
41 to 50yrs	10	28.6 %	3	25.0 %	0	.0%	13	26.0 %	
51 to 60yrs	14	40.0 %	5	41.7 %	2	66.7 %	21	42.0 %	
61 to 70yrs	5	14.3 %	2	16.7 %	1	33.3 %	8	16.0 %	
71yrs & above	1	2.9%	1	8.3%	0	.0%	2	4.0%	

Chi-square test

GFR(ml/min)

Sex	Less than 15		15 to 29		30 to 59		Total		Statistical inference
	(n=35)	*(100 %)*	*(n=12)*	*(100 %)*	*(n=3)*	*(100 %)*	*(n=50)*	*(100 %)*	
Male	24	68.6 %	10	83.3 %	3	100.0 %	37	74.0 %	X^2=2.133 Df=2 .344>0.05 Not Significant
Female	11	31.4 %	2	16.7 %	0	.0%	13	26.0 %	

Chi-square test

GFR(ml/min)

Weight(Kg)	**Less than 15**		**15 to 29**		**30 to 59**		**Total**		**Statistical inference**
	(n=35)	***(100 %)***	***(n=12)***	***(100 %)***	***(n=3)***	***(100 %)***	***(n=50)***	***(100 %)***	
40 to 49kgs	9	25.7 %	1	8.3%	0	.0%	10	20.0 %	X^2=14.887 Df=8 .061>0.05 Not Significant
50 to 59kgs	13	37.1 %	3	25.0 %	0	.0%	16	32.0 %	
60 to 69kgs	8	22.9 %	3	25.0 %	3	100.0 %	14	28.0 %	
70 to 79kgs	5	14.3 %	4	33.3 %	0	.0%	9	18.0 %	
80kgs & above	0	.0%	1	8.3%	0	.0%	1	2.0%	

Chi-square test

GFR(ml/min)

DM	**Less than 15**		**15 to 29**		**30 to 59**		**Total**		**Statistical inference**
	(n=35)	***(100 %)***	***(n=12)***	***(100 %)***	***(n=3)***	***(100 %)***	***(n=50)***	***(100 %)***	
Absent	35	100.0 %	12	100.0 %	3	100.0 %	50	100.0 %	-

Chi-square test

GFR(ml/min)

SHT	Less than 15		15 to 29		30 to 59		Total		Statistical inference
	(n=35)	*(100 %)*	*(n=12)*	*(100 %)*	*(n=3)*	*(100 %)*	*(n=50)*	*(100 %)*	
Absent	12	34.3 %	1	8.3%	2	66.7 %	15	30.0 %	X^2=4.909 Df=2 .086>0.05 Not Significant
Present	23	65.7 %	11	91.7 %	1	33.3 %	35	70.0 %	

Chi-square test

GFR(ml/min)

ECG	Less than 15		15 to 29		30 to 59		Total		Statistical inference
	(n=35)	*(100 %)*	*(n=12)*	*(100 %)*	*(n=3)*	*(100 %)*	*(n=50)*	*(100 %)*	
No	1	2.9%	1	8.3%	1	33.3 %	3	6.0%	X^2=4.703 Df=2 .095>0.05 Not Significant
Ab	34	97.1 %	11	91.7 %	2	66.7 %	47	94.0 %	

Chi-square test

GFR(ml/min)

on dialysis	**Less than 15**		**15 to 29**		**30 to 59**		**Total**		**Statistical inference**
	(n=35)	***(100 %)***	***(n=12)***	***(100 %)***	***(n=3)***	***(100 %)***	***(n=50)***	***(100 %)***	
No	31	88.6 %	9	75.0 %	3	100.0 %	43	86.0 %	X^2=1.887 Df=2 .389>0.05 Not Significant
Yes	4	11.4 %	3	25.0 %	0	.0%	7	14.0 %	

Chi-square test

GFR(ml/min)

Homocysteine (umol/lt)	**Less than 15**		**15 to 29**		**30 to 59**		**Total**		**Statistical inference**
	(n=35)	***(100 %)***	***(n=12)***	***(100 %)***	***(n=3)***	***(100 %)***	***(n=50)***	***(100 %)***	
Negative	7	20.0 %	3	25.0 %	1	33.3 %	11	22.0 %	X^2=.369 Df=2 .831>0.05 Not Significant
Positive	28	80.0 %	9	75.0 %	2	66.7 %	39	78.0 %	

Paired Samples 't'-test

Item	Mean	S.D	Mean	S.D	T	Df	Statistical inference
GFR(ml/min) (n=50)	12.8300	6.95518	-6.8440	11.46562	-4.221	49	.000<0.05 Significant
Homocysteine(umol/lt)(n=50)	19.6740	7.83157					

DISCUSSION

Recently disulfur aminoacid homocysteine has gained much importance because of its role in vascular thrombosis and genesis of atherosclerosis . Chronic kidney disease is also very much prevalent in the general population . The patients with CKD are very much susceptible to cardiovascular system involvement related morbidity and mortality. As recent studies have shown an increased prevalence of hyperhomocysteinemia in CKD patients we tried to conduct a study on this .

Recent articles and publication in internet shows that chronic kidney disease is associated with hyperhomocysteinemia and significantly contributes to cardiovascular morbidity and mortality .

Menon et al , in their study found that hyperhomocysteinemia was prevalent in 56% 0f CKD patients they studied , and hyperhomocysteinemia was partly amenable to treatment with vitamins in stages 3 & 4

In this sudy we found that 78% of CKD patients were having hyperhomocysteinemia correlating with other studies conducted elsewhere in the world and hyperhomocysteinemia was more prevalent as stages of CKD increases . Even though our study sample size was smaller we found

that hyperhomocysteinemia was more prevalent in later stages of CKD. It was in accordance with the concept that as renal function deteriorates the homocysteine excretion decreases and its level increases in plasma .

We even noted in the study that even if the patient was on dialysis it was not affecting the homocysteine level elevation as mentioned in other review literature that homocysteine level will transiently decreases after a dialysis session but swings back to normal within two to three days to predialysis value .

We observed that majority of patients with CKD had some kind of ECG abnormality correlating well with statement that cardiovascular morbidities are most important cause of mortality in patients with CKD . Our main concern to evaluate for the presence or absence of hyperhomocysteinemia in CKD patients was to decrease the cardiovascular morbidity and mortality . So its worthy to take measures to decrease homocysteine levels in patients with CKD .

We tried in all ways to exclude confounding factors like diabetes , smoking , chronic alcoholism, chronic liver disease which are independently associated with hyperhomocysteinemia .

Regarding the sexual difference in hyperhomocysteinemia any definite conclusion cannot be drawn as there are discrepancies among the sample size and sex proportion

We could not exclude the other genetic variations which may affect the homocysteine level . We even could not measure the levels of Vit B12 and Folic acid , Pyridoxine level in patients with CKD due to financial restriction .

Levels of Vitamin B12 and Folic acid in CKD patients to correct hyperhomocysteinemia is more as compared with patients without CKD . So it is advisable to supplement more amount of vitamins to correct hyperhomocysteinemia in CKD patients to prevent cardiovascular morbidity and mortality.

Further studies involving larger sample size with measurement vitamin levels along with genetic studies are required to exactly elucidate the incidence and prevalence of hyperhomocysteinemia in Chronic kidney disease patients and large cohort studies to demonstrate exact correlation between hyperhomocysteinemia and cardiovascular morbidity and mortality and treatment strategy aimed at decreasing hyperhomocysteinemia to reduce cardiovascular morbidity and mortality is necessary

LIMITATIONS OF THIS STUDY

1. Limited sample size
2. Levels of Vitamin B12 and folic acid are not measured
3. Sample distribution is not uniform among CKD patients so as to exactly correlate between decreased renal function and level of hyperhomocysteinemia
4. Genetic aspects of disease not emphasized much

CONCLUSION

Hyperhomocysteinemia was observed in 78 % of patients with CKD

Prevalence of hyperhomocysteinemia was more in End stages of CKD (stage 3 & 4 &5)

Homocysteine elevation was found in both CKD without dialysis and with intermittent hemodialysis or peritoneal dialysis. 94.87 % of patients with CKD were found to have mild hyperhomocysteinemia and 5.1% had moderate hyperhomocysteinemia and none had severe hyperhomocysteinemia

Eventhough we observed hyperhomocysteinemia in 78 % of patients we tested ,further studies involving larger sample size and excluding other risk factors for hyperhomocysteinemia is required to validate this results .

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Proforma for dissertation on chronic kidney disease and plasma homocysteine level

Name : age : sex: IP Number:

address

Weight : Height :

Presenting complaints :

Family history of kidney diseases: yes /no

Whether patient is on dialyasis : yes /no

Whether patient is k/c/o : SHT / Diabetes / CAHD / Chronic liver disease /

alcoholic /smoker

Treatment/drugs patient receiving :

Examination findings

Gneral physical examination :

vitals :

Systemic examination

Cardiovascular system

Respiratory system

Abdomen

Central nervous system

Investigation :

	Day 1	Day 2	Day 3
RBS			
BLOOD UREA			
SERUM CREATINE			
Na+			
K+			
COMPLETE HEMOGRAM			
LIVER FUNCTION TEST			

URINE ROUTINE

ECG

USG ABDOMEN

PLASMA HOMOCYSTEINE

GLOMERULAR FILTRATION RATE (calculated)

CONSENT FORM

I _____ hereby give consent to participate in the study conducted by **Dr.ADARSHA.G.K ,** post graduate in department of internal medicine ,Thanjavur medical college & hospital, Thanjavur – 613004 and to use my personal clinical data and result of investigation for the purpose of analysis and to study the nature of disease. I also give consent for further investigations

Place :

Date : Signature of participant

S.NO	Name	IP NO.	Age(years)	Sex	Weight(Kg)	DM	SHT	Urea(mg%)	Creatinine(mg%)	ECG	on dialysis	GFR(ml/min)	Homocysteine(u	
1	kathayee	32296	60	F	45	A	A	102	5.5	Ab	N	7.72	14.63	
2	seeman raj	40955	35	M	50	A	A	160	9.8	Ab	N	7.44	15.57	
3	kaliyaperumal	40814	60	M	60	A	P	100	3.7	Ab	N	16.57	21.72	
4	anjalai	40246	58	F	60	A	P	150	9.2	Ab	N	12.6	24.45	
5	kannan	39376	50	M	50	A	P	150	9.2	Ab	Y	7.35	18.5	
6	kaliyaperumal	38185	47	M	60	A	P	150	13.8	Ab	Y	4.93	15.45	
7	kamalambal	37967	75	F	75	A	P	150	6.4	Ab	N	9.4	12.27	
8	selvaraj	40244	55	M	75	A	P	96	6.9	Ab	N	13.02	33.58	
9	palanisamy	40251	64	M	70	A	A	128	10.8	Ab	N	6.84	22.17	
10	siddique	39539	34	M	70	A	P	154	15.7	Ab	N	6.77	11.42	
11	perumal	40233	70	M	70	A	P	87	5	Ab	N	15.55	23.71	
12	elangovan	37484	54	M	60	A	P	138	8.8	Ab	N	7.96	18.51	
13	parimalam	38113	44	F	50	A	A	112	9.9	N	N	6.73	16.23	
14	panduranga	41327	60	M	50	A	P	88	3.6	Ab	N	15.43	10.58	
15	kalaimani	40056	28	F	60	A	P	142	12.8	Ab	N	6.36	26.98	
16	ravichandran	40844	52	M	100	A	P	108	4.7	Ab	N	26	9.62	
17	paramayyan	40895	48	M	50	A	A	70	8.1	Ab	Y	8.24	17.43	
18	manimegalai	40465	55	M	45	A	A	94	5	Ab	N	10.06	17.85	
19	ramaraj	39796	51	M	45	A	A	88	4.5	Ab	N	12.36	21.99	

S.NO	Name	IP NO.	Age(years)	Sex	Weight(Kg)	DM	SHT	Urea(mg%)	Creatinine(mg%)	ECG	on dialysis	GFR(ml/min)	Homocysteine(u	
20	mathivanan	40209	61	M	60	A	P	78	2	Ab	N	33.46	13.86	
21	ramaiyan	30076	60	M	45	A	P	84	3.2	Ab	N	15.62	18.28	
22	govindaraj	38846	60	M	60	A	A	60	2	Ab	N	30.48	18.17	
23	ayyakannu	38878	55	F	40	A	A	88	3.8	Ab	N	12.42	27.94	
24	selvaraj	41466	44	M	65	A	P	132	10.5	Ab	Y	8.25	25.28	
25	selvaraj	41354	58	M	60	A	P	180	14	Ab	Y	15.2	24.91	
26	Gandhi	42076	48	F	45	A	P	148	8.3	Ab	N	6.92	19.88	
27	ummusulma	41758	55	F	68	A	P	130	8.8	Ab	N	9.14	24.14	
28	Manobalan	42008	18	M	52	A	P	172	22.2	Ab	N	3.96	22.36	
29	Kaveri	41914	66	F	70	A	P	120	3.9	Ab	Y	18.4	16.76	
30	Muthusamy	83714	68	M	45	A	P	78	3.2	Ab	N	14.61	15.58	
31	Murugaiyan	83814	65	M	50	A	P	74	3.9	Ab	N	13.35	14.44	
32	Soundarraj	41924	52	M	54	A	A	132	12.6	Ab	N	5.36	13.27	
33	Alagammal	41984	54	F	50	A	P	204	20.5	Ab	N	2.43	29.08	
34	Thangarasan	41850	65	M	58	A	P	122	4.3	Ab	N	14.05	18.24	
35	Rajendran	40015	52	M	52	A	A	92	5	Ab	N	11.6	22.8	
36	Rajendran	40845	51	M	50	A	A	120	6.8	Ab	N	13.1	26.18	
37	Ramasamy	32323	50	M	60	A	P	114	6.8	Ab	N	11.1	19.58	
38	Veearmay	43346	47	M	60	A	P	112	2.9	Ab	Y	26.72	16.01	

S.NO	Name	IP NO.	Age(years)	Sex	Weight(Kg)	DM	SHT	Urea(mg%)	Creatinine(mg%)	ECG	on dialysis	GFR(ml/min)	Homocysteine(u	
39	Muthu	48346	60	M	60	A	A	60	2.7	N	N	30	17.44	
40	Rangasamy	40386	47	M	48	A	P	114	9.8	Ab	N	6.6	15.32	
41	Jayakanndan	39253	50	M	50	A	P	88	4.3	Ab	N	15.9	24.69	
42	Sanyasimuthu	40392	75	M	75	A	P	88	3.8	Ab	N	17.8	19.53	
43	Thangaponnu	29766	50	F	48	A	P	80	3.8	Ab	N	13.41	17.09	
44	Subramaniyam	29876	48	M	75	A	P	100	4.5	Ab	N	21	9.82	
45	kaliyaperumal	40392	62	M	78	A	P	98	8.4	Ab	N	10.05	58	
46	Rajagopal	17152	60	M	45	A	P	93	4.9	Ab	N	10.2	15.16	
47	Kathaiyan	36927	51	M	58	A	P	110	8.1	Ab	N	13.5	9.34	
48	Rani	30987	40	F	50	A	A	84	3.4	N	N	17.9	18.15	
49	Duraikannu	30894	49	M	55	A	P	135	9.3	Ab	N	9.47	12.11	
50	Renugadevi	40025	34	F	60	A	A	126	10.8	Ab	N	8.17	27.63	

F	FEMALE
M	MALE
A	ABSENT
P	PRESENT
Y	YES
N	NO